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ASSESSING THE IMPACT OF MARINE SEISMIC SURVEYS ON SOUTHEAST AUSTRALIAN SCALLOP AND LOBSTER FISHERIES

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Abbreviations

BI Bundle index = ([egg bundle mass · body mass^{-1}] · 100)

CL Carapace length

HPI Hepatopancreas index = ([hepatopancreas mass · body mass^{-1}] · 100)

SEL Sound exposure level

SEL_{cum} Cumulative sound exposure level

THC Total haemocyte count

Executive Summary

The present study, undertaken by University of Tasmania's Institute for Marine and Antarctic Studies in conjunction with Curtin University's Centre for Marine Science and Technology, was developed to investigate the potential impact of seismic surveys on economically important fishery species. Substantial overlap exists between important fishing grounds and areas of interest for oil and gas exploration within southeast Australian waters. The fishing industry is now very concerned about the potential of intense low frequency acoustic signals produced during these surveys to disturb, harm or even kill fisheries species. Studies conducted to date generally report that fish can demonstrate behavioural responses to seismic activities, including startle and flight responses, displacement, dispersal, and disruption of feeding or breeding activity. These behavioural responses could in turn result in changes in commercial catch rates. There have been very few dedicated studies of the effects of marine seismic surveys on invertebrates, and the limited information on invertebrates suggests that they may be relatively resilient to seismic sound, however, further research is required before the impacts of seismic activity on commercially important invertebrates can be dismissed. In the light of a general lack of well-designed and scientifically rigorous studies examining the effect of marine seismic surveys on invertebrates and in the absence of any detailed specific studies on commercial scallops (*Pecten fumatus*) and southern rock lobster (*Jasus edwardsii*), fishers in Victoria and Tasmania have lobbied for dedicated research targeting these valuable resources. This study aimed to use a field and laboratory experimental approach to determine the impact of marine seismic surveys on these important fisheries species. The results obtained are broadly applicable to scallop and spiny lobster fisheries throughout the world, and bivalve and crustacean fisheries in general.

Objectives

Specifically, this study aimed to: 1) determine the impact of intense low frequency acoustic signals on adult southern rock lobsters, including berried (egg carrying) females; 2) determine the impact of intense low frequency acoustic signals on adult commercial scallops; and 3) estimate exposure levels required to produce observed biological impacts from marine seismic surveying.

Methods

To address these objectives, an extensive exposure regime was carried out using an industry standard air gun in a field setting chosen to emulate the natural habitats of lobsters and scallops, respectively. The air gun, a Sercel G Gun II, was fitted with either a 45 in^3 or a 150 in^3 chamber to conduct four exposure experiments in lobsters and three exposure experiments in scallops. Following exposure, a total of 302 lobsters, the majority of which were berried females, were sampled. Experiments generally consisted of four sampling times between days 0 and 120 post-exposure though in one experiment a group of animals were maintained and assessed over 365 days post-exposure. At each sample time, lobsters were assessed for mortality, two behavioural reflexes, damage to the primary mechanosensory organ (balance and gravity sensing organ, similar to the human inner ear), condition, biochemistry of the haemolymph (i.e. blood analogue), the number of circulating haemocytes (i.e. blood cell analogues) and embryo development. Each lobster experiment comprised two treatments, a control pass of the air gun where it was deployed but not operated, and an active pass of the air gun. A total of 560 scallops were sampled at three time points

between days 0 and 120 post-exposure for mortality, haemolymph (blood analogue) biochemistry, the number of circulating haemocytes (blood cell analogues), righting reflex, recessing behaviour and condition indices. Each scallop experiment comprised four treatments, a control pass of the air gun deployed but not operated, one pass of the air gun, two passes of the air gun or four passes of the air gun.

Key findings in seismic air gun exposure

At long range (i.e. greater than 100 m) sound transmission was different at the lobster site compared to the scallop site. The lobster site was a hard limestone reef platform which acted to increase sound transmission losses compared to the sandy scallop site. At short range (i.e. less than 100 m), there was little difference in transmission loss at either site. Using modelling of a commercial seismic source, the waterborne air gun sound exposure levels during lobster experimental exposures from the single 150 in^3 air gun emulated passage of a large air gun array operating in 30-100 m water depth passing within 200-500 m range of the test animals. The geophone data revealed energy travelling through the seabed as expected but also a high amplitude 'shaking' of the seabed lasting for ~ 70 ms, which was important for the stimuli involved in scallop impacts. The scallops were directly coupled to the seabed so would have experienced all of this 'shaking'. The ground motion was believed derived from interface waves in the sediment excited by the air gun signal plus the direct ground stimulation by the air gun signal's waterborne sound particle motion. This ground motion had an outlying ground roll acceleration maximum magnitude measure of 68 ms^{-2} although short range measures typically fell within the range 3-20 ms^{-2} (± standard deviation about the mean) for the single air gun within 100 m range. Modelling a commercial seismic source gave comparative ranges for exposure measures of maximum single shot sound exposure level, cumulative sound exposure level and ground motion during scallop experiments, equivalent to the seismic source passing at 114-875 m range for the single pass, 114-500 m range for two air gun passes or 115-275 m for the third regime of four air gun passes. In any experimental regime, if the commercial air gun array passed at shorter ranges than as listed, exposures would have been higher than as experienced during experiments.

Key findings in lobsters

Seismic exposure did not result in any lobster mortality over the course of any of the four experiments. However, a range of sub-lethal effects were observed. Reflexes were measured to assess the potential for impairment of neurological control of the body and any impact on the ability of the lobster to control its positioning. First, a simple reflex, tail extension, was assessed. In the three winter experiments (winter 2013, 45 in^3 air gun operated at standard pressure; the winter 2014, 150 in^3 air gun operated at low pressure; and the winter 2014, 150 in^3 air gun operated at standard pressure), no difference was found between control lobsters and exposed lobsters. In the summer 2015, 150 in^3 air gun standard pressure experiment, exposure significantly reduced the ability of lobsters to maintain tail extension. Immediately after exposure (day 0), lobsters exposed to air gun signals showed a 32% decrease in tail extension compared to control lobsters that were not exposed. This response persisted to 14 days after exposure, when exposed lobsters had a 23% decrease in the ability to maintain tail extension. The effects of stress in lobsters are known to be exacerbated in warm summer conditions, which explains why this response was only observed in the experiment conducted in the summer. However, the duration of the response indicates that its cause cannot be

explained simply by fatigue and the cause is more complex. The disruption of the capacity for tail extension, which is a simple reflex requiring little neural control, suggests that more complex reflexes and behaviours, such as escaping from a predator, may be impacted, although the ecological implications were not investigated in this study.

The second reflex evaluated was the righting response, a complex reflex. To assess this reflex, the time lobsters took to right themselves after being placed on their back was measured and compared. Exposure was found to significantly increase righting time in three of the four experiments. In the winter 2013, 45 in^3 standard pressure experiment, exposure more than doubled righting time, increasing it by 157%, from an average range of 1.7-3.4 seconds in control lobsters to an average range of 5.7-8.2 seconds in exposed lobsters over the course of the 120 days of the experiment. In the winter 2014, 150 in^3 low pressure experiment, similar results were found, with control lobsters righting in 1.4-2.9 seconds compared and exposed lobsters taking 120% longer, with an average that ranged from 4.2-5.5 seconds, with slowed righting persisting for 365 days post exposure and after a moult. In the summer 2015 experiment, exposed lobsters were again slower, taking 80% longer to right over the 14 days of the experiment. Interestingly, in the winter 2014, 150 in^3 standard pressure experiment, no difference in righting time was found between treatments.

To better understand the righting results, the primary mechanosensory organ, the statocyst, was investigated. The statocysts are a pair of fluid-filled sacs found at the base of the antennules. These organs are similar to the vestibular canal system of the human inner ear, and are filled with sensory hair cells that detect gravity and body position. Comparing the hair cells between treatments showed significant damage in the exposed treatments from the winter 2013, 45 in^3 standard pressure, winter 2014, 150 in^3 low pressure and summer 2015, 150 in^3 standard pressure experiments. Statistical analysis showed that this damage was correlated to impaired righting time, with greater damage resulting in slower righting. In the winter 2014, 150 in^3 low pressure experiment, this damage was found to persist to 365 days post-exposure, after lobsters had moulted, indicating that this damage may be permanent.

In the winter 2014, 150 in^3 standard pressure experiment, in which no difference was observed in righting time between treatments, control lobsters not exposed to air gun signals were found to show levels of damage similar to that of the exposed lobsters in the other experiments. Exposure in this experiment did not result in additional damage. The lobsters in this experiment were obtained from a site subject to higher levels of anthropogenic environmental noise such as vessel noise from large cargo ships and smaller recreational boats as well as low frequency noise possibly associated with localised pumping systems. The lobster population at this site is extensively monitored and is thriving, making the ecological implications of statocyst damage, in particular the pre-existing environmental damage, unclear. It also raises the possibility that the lobsters are able to adapt to statocyst damage, as these lobsters did not display impaired righting reflexes.

To understand the physiological effect of exposure, several assays of lobster haemolymph were performed. Haemolymph is the invertebrate analogue to vertebrate blood, and carries out many of the same functions, including transport of oxygen, waste and nutrients and mediating immune response. The first haemolymph parameter examined was its biochemistry. Neither haemolymph pH nor assays of 23 electrolyte and mineral ions (e.g. Na, Cl, Mg, Ca, etc.), organic molecules (e.g. glucose, lactate, triglycerides, etc.) and enzymes (amylase, lipase, aminotransferases, etc.) showed a

response to exposure, indicating that lobsters are physiologically resilient to air gun signal exposure. The refractive index of the haemolymph, a measure of nutritional condition, indicating how well lobsters are able to consume, digest and assimilate food, showed a response in one experiment, the winter 2014, 150 in^3 low pressure experiment. At the 120 and 365 day post-exposure points in this experiment, exposed lobsters had significantly reduced refractive index, indicating a reduced nutritional status. However, this result was not found in any of the other three experiments and no other condition indicators suggested the exposed lobsters were negatively affected.

The final analysis of haemolymph was counts of the number of circulating haemocytes, an analogue to blood cells that function in immune response and are often used as an indicator of health. Haemocyte counts showed a significant response to exposure in all four experiments, with exposure resulting in a reduction in cell numbers. In the winter 2014 150 in^3 low pressure experiment, this reduction was progressive over time, reaching a low point at 120 days post exposure. Decreases in circulating haemocytes typify the response to trauma or stress and frequently leave the lobster vulnerable to infection. Furthermore, in that same experiment, exposed lobsters maintained until 365 days post exposure showed a 100% increase in haemocyte count over control lobsters, potentially indicating an immune response to pathogens. These results raise some concern that exposure may affect the immune system of lobsters over a chronic (months post-exposure) time period, leaving them vulnerable to pathogens. The lobsters in this study did not show any visible signs of infection and no mortality was observed, however, they were maintained in laboratory conditions. Further study is required to evaluate whether immune function is altered and if there is any impact to animals in the wild.

To evaluate whether exposure affected the development of lobster embryos following exposure early in embryonic development, the berried (egg-bearing) female lobsters were maintained until the eggs hatched. Hatched larvae were found to be unaffected in terms of egg development, the number of hatch larvae, larval dry mass and energy content and larval competency (i.e. survival in adverse conditions). In the winter 2013, 45in^3 standard pressure experiment, a slight but significant difference was found in larval length, with exposed larvae 1.5% longer. This difference is unlikely to be biologically relevant, as it is well within the range of natural variation in embryo length. These results suggest that exposure during the early embryonic stage did not impair the development and hatching of lobster larvae.

Key findings in scallops

Immediate mass mortality of scallops in response to air gun exposure was not observed and overall mortality rates in all three experiments were at the low end of the range of the naturally occurring mortality rate documented in the wild, which ranges from 11-51% with a 6 year mean of 38%. However, increases in the level of exposure (i.e. repeated exposure to air gun passes) were found to significantly increase mortality. This increased rate of mortality manifested over the 120 days post-exposure of this experiment, with the risk of mortality increasing significantly over time and the majority of mortalities, *ca.* 60% recorded at the 120 day sample point. Compared to control scallops, which were found to have a total mortality rate of ≤5% in all three experiments at day 120 post-exposure, exposed scallops showed mortality rates of 9%-11% in the 1-pass treatment, 11%-16% in the 2-pass treatment and 15%-20% in the 4-pass treatment in the three experiments comprising this study. In the summer, 2015 experiment, both control and exposed treatments suffered complete

mortality at some point after the day 14 sample point and prior to the day 120 sample point, which was not related to seismic exposure.

Scallop haemolymph is responsible for a number of functions, including oxygen and nutrient transfer, waste removal and immune response and is used as an indicator of health and stress response. The number of circulating haemocytes were compared between treatments, with two different patterns of response observed. In the 2013 experiment, exposed scallops showed a significant reduction in haemocytes compared to controls immediately after exposure at day 0. At day 14, no differences were observed between controls and any of the exposed scallops. In the 2014 experiment, no difference was observed at day 0 and at day 14, exposed treatments showed higher levels of haemocytes than controls. Haemocyte counts were only recorded at day 14 in the 2015 experiment, with results similar to those of the 2014 experiment at that sample point, with significantly more circulating haemocytes in the exposed treatments than in the controls. At day 120, both the 2013 and 2014 experiments showed similar results, with haemocyte numbers in exposed treatments collapsing to a level around half that of control scallops. The difference in response at the early sample times can be explained by differences in collection methods and the influence of other stressors. For the 2013 experiment, scallops were obtained via dredging, whereas in 2014 and 2015, scallops were hand collected by divers. The stress involved with dredging resulted in a depressed haemocyte count in the 2013 scallops, with seismic exposure acting in synergy, raising concern over how seismic exposure may interact with other stressors (e.g. predation, fishing, ocean temperatures, etc.). The collapse in haemocyte numbers in exposed scallops at the day 120 sample point in both 2013 and 2014 indicates chronic depression of haemocytes and a high likelihood that affected scallops were immunocompromised, one of the leading causes of mortality events.

Haemolymph biochemistry showed a trend of alkalosis (increased pH) in exposed scallops at days 0 and 14 in the 2014 experiment and at day 14 in the 2015 experiment. Reports of alkalosis in marine invertebrates are rare and in the exposed scallops in the present study, alkalosis persisted for much longer and under substantially different environmental conditions compared to previous reports. Based on these differences, it is not possible to characterise the mechanisms underpinning this response, though damage to gill tissue has been advanced as a hypothesis requiring further study.

A range of eight haemolymph electrolyte and mineral ions assayed showed a significant response to exposure, with sodium, potassium, calcium and chloride showing overall trends of increasing concentration with repeated exposure and magnesium and bicarbonate showing decreasing concentration in response to exposure. Protein and glucose levels in the haemolymph also decreased with exposure. Other metabolites, organic molecules and enzymes showed no change. The disruption of the ability to control the concentration of electrolytes and minerals in the haemolymph indicates a severely compromised physiology, particularly as the impact persisted over the course of the entire experiment (day 120 post-exposure). Investigating the cause of these imbalances was not within the scope of this study, though damage to kidney and/or gill tissues is hypothesised and require further investigation. The ecological implications of these extreme physiological changes also warrant further study, as they may have substantial impacts on the ability for scallops to cope with further stressors (e.g. dredging, temperature changes, etc.) in the wild, following exposure to seismic signals.

Scallop behaviour was also altered by exposure to air gun signals, with a decrease in classic behaviours – including positioning, mantle irrigation and swimming – and the elicitation of a novel flinching behaviour observed exclusively during exposure at a range of up to 350 m from the air gun source. Despite this change in behaviour, an adoption of energetically expensive behaviours (e.g. swimming, extensive valve closure) was not observed, with comparisons of tentacle extension, used as a proxy to indicate valve closure, showing no difference before, during or after exposure. Similarly, swimming was observed only rarely and was not in response to exposure. Comparison of the rate in which scallops recess themselves into the sediment, which is considered their "natural" state, showed that the number of air gun passes scallops were exposed to corresponded to an increase in recessing rate, resulting in a stepwise, dosage dependent response with scallops receiving the highest level of exposure recessing the fastest and control scallops recessing the slowest. The righting reflex, on the other hand, was the fastest in control scallops and significantly slower in exposed scallops. The ecological implications of these changes in behaviour and reflexes require further study, as they may have substantial impacts on the ability for scallops to cope with predators in the wild.

A range of condition indices showed some minor differences between treatments. Relative tissue mass showed differences between treatments in the 2013 and 2014 experiments, though there was no consistent trend in the response. In the 2015 summer experiment, control scallops demonstrated a significantly higher percent adductor mass and relative adductor mass than exposed scallops. These results suggest that somatic tissue and adductor mass are largely unaffected by seismic exposure, though there is some indication that that scallops may be affected by seismic exposure during the warm water conditions in summer.

Implications

Seismic surveys appear to be unlikely to result in immediate large scale mortality in the southern rock lobster fishery and, on their own, do not appear to result in any degree of mortality. Furthermore, early stage lobster embryos showed no effect from air gun exposure, indicating that at this point in life history, they are resilient to exposure and subsequent recruitment should be unaffected. We did not conduct experiments on the full suite of lobster embryonic and larval stages so we cannot predict how seismic signals impact lobster larval success beyond exposure during early embryo development. Exposure to air gun signals resulted in alteration to several important aspects of adult lobster biology, including depression of the number of haemocytes available for immune response, impairment of reflex behaviours involved with tail control and righting, and damage to the sensory hairs of the statocyst.

In considering the severity of these effects, it is necessary to keep in mind that this study did not investigate ecological impacts. For example, is it not possible to determine whether the reduced number of haemocytes might compromise the immunity of lobsters in the wild, rather than in well maintained tanks receiving filtered seawater. It is also not possible to conclude how the observed impairment of reflexes or damage to the balance organ might translate to the complex environment in the wild, such as reducing the ability to avoid predators or compete with other lobsters for food or reproduction. Until the full scope of these changes and their ecological effects can be more thoroughly investigated, caution must be taken against extrapolating the results of this study to situations that were not within its scope.

In scallops, seismic exposure did not cause immediate mass mortality, however, exposure, particularly repeated exposure, increased the risk of mortality significantly and scallops showed severely compromised physiology over a chronic (4 months) time frame from which there were no signs of recovery. There were also significant changes in behaviour and reflexes during and following seismic exposure. Given the compromised physiological condition of the exposed scallops in this study it is likely that they would have reduced tolerance to subsequent stressors, including environmental, nutritional and pathological stressors. Furthermore, it is presently unclear whether the observed physiological impairment would result in heightened chronic mortality in timeframes beyond those examined in the current study. An extended study, along with a better understanding of the mechanisms underlying the considerable physiological disruption observed is necessary to fully understand the ultimate outcomes resulting from exposure to air gun signals. It is also important to note that this study investigated adult scallops only and did not cover any aspect of reproduction or embryonic, larval, or juvenile life stages.

Keywords

seismic survey, air gun, spiny lobster, rock lobster, *Jasus edwardsii*, scallop, *Pecten fumatus*, statocyst, haemocyte, osmoregulation

Introduction

Anthropogenic noise has shown the potential to negatively affect animals from arthropods to mammals through the disruption of fundamental biological processes such as metabolism, immune function, reproduction and development (Kight & Swaddle 2011). The impacts of anthropogenic noise in aquatic environments are of particular concern (Turnpenny *et al.* 1994; Popper & Hastings 2009; Slabbekoorn *et al.* 2010) as sound travels farther, faster and more efficiently (i.e. lower attenuation of intensity) in water than through air (Berg & Stork 2005), resulting in a greater area of potential impact.

A significant source of anthropogenic noise in the marine environment is the use of seismic air guns for oil and gas exploration. Air guns represent a technological advancement offering an apparent improvement in animal welfare over the effects of previous methods, such as explosives, which show a distance dependent spectrum of impact ranging from mortality at close range to organ damage, sensory disruption and behavioural alterations at increasing distances from the source (Keevin & Hempen 2004). Nonetheless, the acoustic signals produced by air guns are high energy, intense and primarily low frequency, reaching theoretical source levels of up to 260 dB re 1 μPa rms @ 1m in the downward direction across a 10-200 Hz bandwidth (Hildebrand 2009). It needs to be noted that these theoretical source levels are never actually reached with real spatially distributed sources and levels ~ 30-40 dB less may be expected near commercial air gun arrays. Such high impulse levels raise concerns over the effects of air gun signals on wildlife, as marine mammals (Gordon *et al.* 2004) and fishes (Pearson *et al.* 1992; Wardle *et al.* 2001; Kastelein *et al.* 2008; Fewtrell & McCauley 2012) have been shown to demonstrate altered behaviour and physiology following exposure. Economic concerns have also been raised over reduced abundance and catch rates reported during and immediately following seismic surveys for a variety of fisheries species, e.g. blue whiting *Merlangus merlangus* (Dalen & Knutsen 1986), rockfish *Sebastes* spp. (Skalski *et al.* 1992), cod *Gadus morhua* and haddock *Melanogrammus aeglefinus* (Engås *et al.* 1996), herring *Clupea* spp. (Slotte *et al.* 2004), American lobster *Homarus americanus* (Payne *et al.* 2007) and snow crab *Chionoecetes opilio* (Christian *et al.* 2003).

Despite their ecological and socioeconomic importance, comparatively little is known about the impact of seismic surveys on marine invertebrates. A recent gap analysis by Hawkins *et al.* (2015) highlighted a range of issues to be addressed before conclusions can be drawn by researchers, industries and regulatory bodies. These issues range from improving the current understanding of the sources of aquatic noise and the methods and metrics used to quantify exposure, to the characterisation of sound propagation through the water, and the ability of marine invertebrates to produce and even sense sound. It is not surprising, given these substantial gaps in knowledge, that industry groups representing commercially important invertebrates such as spiny lobsters (Parry & Gason 2006) and scallops have cited concern over seismic surveys resulting in mass deaths (Parry & Gason 2006; Anon 2010), with one such incident in Bass Strait, Australia blamed by industry groups for the loss of up to 24,000 tonnes of scallops worth an estimated AU$70 million (Tasmania Scallop Fishermen's Association 2011).

Studies of seismic exposure on American lobster (*Homarus americanus*) have shown a limited range of effects, with exposure to a 40 in^3 air gun resulting in reduced haemolymph calcium and protein levels, glycogen deposits in the hepatopancreas and increased food consumption, whereas

mortality, righting time and haemolymph enzyme levels indicative of tissue damage were unaffected (Payne *et al.* 2007, 2008a). A statistical analysis of catch-per-unit-effort (CPUE) data collected over nearly 30 years in the Victorian southern rock lobster (*Jasus edwardsii*) fishery showed no influence of historical seismic survey activity, though the authors noted a lack of sensitivity due to the preponderance of surveys conducted in deep water away from fishing areas and catch rates would have had to decrease by around 50% to detect a result (Parry & Gason 2006). More broadly, an investigation into the effects of seismic exposure on the snow crab (*C. opilio*) showed that exposure to either a single 40 in^3 air gun or a 200 in^3 array had little effect, with mortality; haemolymph biochemistry, including measurements of refractive index, protein concentration, differential haemocyte count; and statocyst condition unaffected (Christian *et al.* 2003). However, the eggs of berried females in that experiment showed an increased mortality rate and delayed development (Christian *et al.* 2003). Conversely, Dungeness crab (*Cancer magister*) zoea (larvae) showed no effect following field-based exposure to air gun signals (Pearson *et al.* 1994).

The effects of air gun exposure on scallops have similarly received little attention. Brand and Wilson (1996, in Parry and Gason 2006) reported a decline in scallop catch rates following repeated seismic survey exposure; however they attributed the decline to poor recruitment in prior years rather than the seismic exposure. Studies conducted by Parry *et al.* (2002) and Harrington *et al.* (2010) reported no evidence of increased mortality or reduced adductor quality in scallops exposed to commercial seismic surveys, though it must be noted that scallops were suspended in lantern nets during exposure in the former study, and as such, were not subject to the ground borne vibrations that would be experienced in a natural setting. On the other hand, laboratory based exposure to aquatic noise approximating (but notably, not emulating) a seismic survey had a catastrophic effect on scallop larvae (*Pecten novazelandiae*), characterised by abnormal morphological development (Aguilar de Soto *et al.* 2013). However, the applicability of these laboratory assessments to in situ seismic surveying is unclear, as acoustic studies conducted in laboratory tanks have been discouraged for half a century (Parvulescu 1967; Popper & Fay 1993; Rogers *et al.* 2016) owing to an inability to understand what stimulus animals in the tank are actually exposed to, a result of the physics of generating signals and long wavelength sound in small, reflective tanks.

Clearly, given the almost complete lack of research; the contradictory results of what little research has been conducted; the change in sensory capability for a species during development; and the considerable diversity within, and substantial differences between, the molluscan phylum and the crustacean subphylum, drawing any sort of conclusion on the developmental, physiological, ecological impacts of exposure to seismic air gun signals on marine invertebrates is not possible. Without a better understanding of the effects and impacts of exposure to seismic air gun signals, evidence based management and regulation decisions may be difficult to make and any claims of financial loss following surveys are impossible to either substantiate or refute.

In light of the substantial gaps in knowledge, the confounding methods employed by previous studies and the subsequent conflicting results, the present study investigates the impacts of seismic air gun exposure on the southern rock lobster (*Jasus edwardsii*) and the commercial scallop (*Pecten fumatus*). The spiny lobster from the family Palinuridae is a useful model for marine invertebrates, as it is the most valuable single species capture fishery in Australia and spiny lobsters are amongst the most valuable fishery species worldwide (Jeffs & Hooker 2000), with an annual catch of over

81,000 tonnes in 90 countries worth an estimated US$775 million (FAO 2014). Scallops also represent an important resource in Australia, with catch rates varying from 279-1418 tonnes in recent years, worth an estimated AUD$0.5-2.5 million (AFMA 2015). Worldwide, scallop fisheries harvest nearly 750,000 tonnes annually comprising 45% of the total bivalve catch and nearly two-thirds (64%) of the total value at US$1.4 billion (FAO 2014).

The current project was developed to assess the effect of seismic exposure on these two socioeconomically important invertebrates through the quantification of a range of physiological and behavioural / parameters. In lobsters, mortality was assessed immediately following exposure and over the course of a range of time periods, with the longest extending to one year post-exposure. The first set of evaluations of sub-lethal effects considered two reflexes, tail extension and righting. Both reflexes are commonly used in crustacean fishery industries as indicators of stress for assessment of vitality, with tail extension representing a simple reflex and righting a complex reflex, requiring neural control of muscular coordination (Stoner 2012). Next, a number of haemolymph parameters were investigated to identify any disruptions of homeostasis, based on the long history of using haemolymph as a primary tool for assessing physiological indicators of environmental and anthropogenic stress in crustaceans (Fotedar & Evans 2011; Stoner 2012). Haemolymph responses were divided into two components, the haemocytic response, quantified through total haemocyte counts, and the humoral response, quantified through the biochemistry of the fluid fraction, including comparisons of pH, refractive index and a range of electrolytes, minerals, organic molecules and enzymes. Nutritional condition, measured using the mass of the hepatopancreas relative to body mass, was investigated to provide an indication of whether exposure affected the ability to access, digest or assimilate food in lobsters. Reproduction was compared to determine whether air gun exposure results in maternal stress (Smith & Ritar 2005), egg mortality, delayed development or severe morphological abnormality (Christian *et al.* 2003; Aguilar de Soto *et al.* 2013), which could compromise reproductive output and reduce recruitment. Finally, the primary mechanosensory organ of lobsters, the statocyst, was examined to determine whether exposure resulted in any damage which may compromise the equilibrium of lobsters.

In scallops, mass mortality has been hypothesised to result following seismic surveys as a result of energetically expensive behaviours, such as extensive swimming or prolonged valve closure, that result in a fatal energy deficit (Harrington *et al.* 2010). To test this hypothesis, mortality rates were assessed through observation at time points ranging from immediately after exposure to four months post-exposure. Sub-lethal effects were quantified through assays of the haemocytic and humoral components of the haemolymph, with measurement of the same parameters as in lobsters based on the similarities in composition and function of haemolymph between the taxa. Although there are few investigations into the various parameters of pectinid haemolymph, bivalve haemolymph physiology and biochemistry has been well studied in regards to haemocyte response to biotic and abiotic stressors (Chen *et al.* 1987; Suresh and Mohandas 1990; Anderston *et al.* 1995; Livingstone *et al.* 2000; Hannem *et al.* 2009; Hannem *et al.* 2010) and regulation of haemolymph biochemistry (Shumway1977; Thompson 1977; Burton 1983; Ford & Paillard 2007). Video recordings of scallops were used to analyse behaviour prior to, during and following exposure to determine whether air gun signals result in behavioural alterations such as the high energy behaviours posited by the mortality hypothesis. Following exposure, scallop reflex was quantified using two reflexes: righting and recessing. Similar to lobsters, comparing righting in scallops consisted of comparing the time taken to return to a ventrum-up position after being placed dorsum-

up. Recessing measured the time taken for scallops to bury themselves into the substrate, a state considered the "natural" position of scallops (Baird 1958; Minchin 2003). Finally, scallop condition was assessed using measurements of total mass, tissue mass and adductor mass to investigate whether exposure resulted in a decrease in the relative proportion of the adductor or soft tissue.

The present study represents one of the most comprehensive and scientifically robust investigations into the effects of seismic surveys on marine invertebrates to date. Specifically, exposure was conducted in the field with animals in a natural habitat using a real air gun to emulate real world seismic surveys. This was followed with a comprehensive suite of physiological and behavioural assays, with animals held in controlled conditions. Most of the experiments were conducted over the course of 120 days post-exposure, with one experiment lasting for 365 days post-exposure. This approach yielded results that can be extrapolated to real world settings and will serve as a standard for subsequent experiments.

Objectives

1. Determine the impact of intense low frequency acoustic signals on adult southern rock lobsters, including berried (egg carrying) females;
2. Determine the impact of intense low frequency acoustic signals on adult commercial scallops;
3. Outline threshold distances for potential impacts of seismic surveying.

General Methods

Field sites

Two study sites were used: a 10-12 m deep, sandy substrate site for scallops, and a 10-12 m deep, limestone rock platform for lobsters. The locations of study sites, the boat ramp used to launch vessels and the Taroona aquaculture facility are shown in Figure 1. Four periods of experiments were conducted, including a pilot study in 2013 and three periods in which repeat experiments were carried out with scallops (three experiments) and lobster (four experiments). Details of vessels used, experiments carried out and details of the air gun source vessel are listed in Table 1

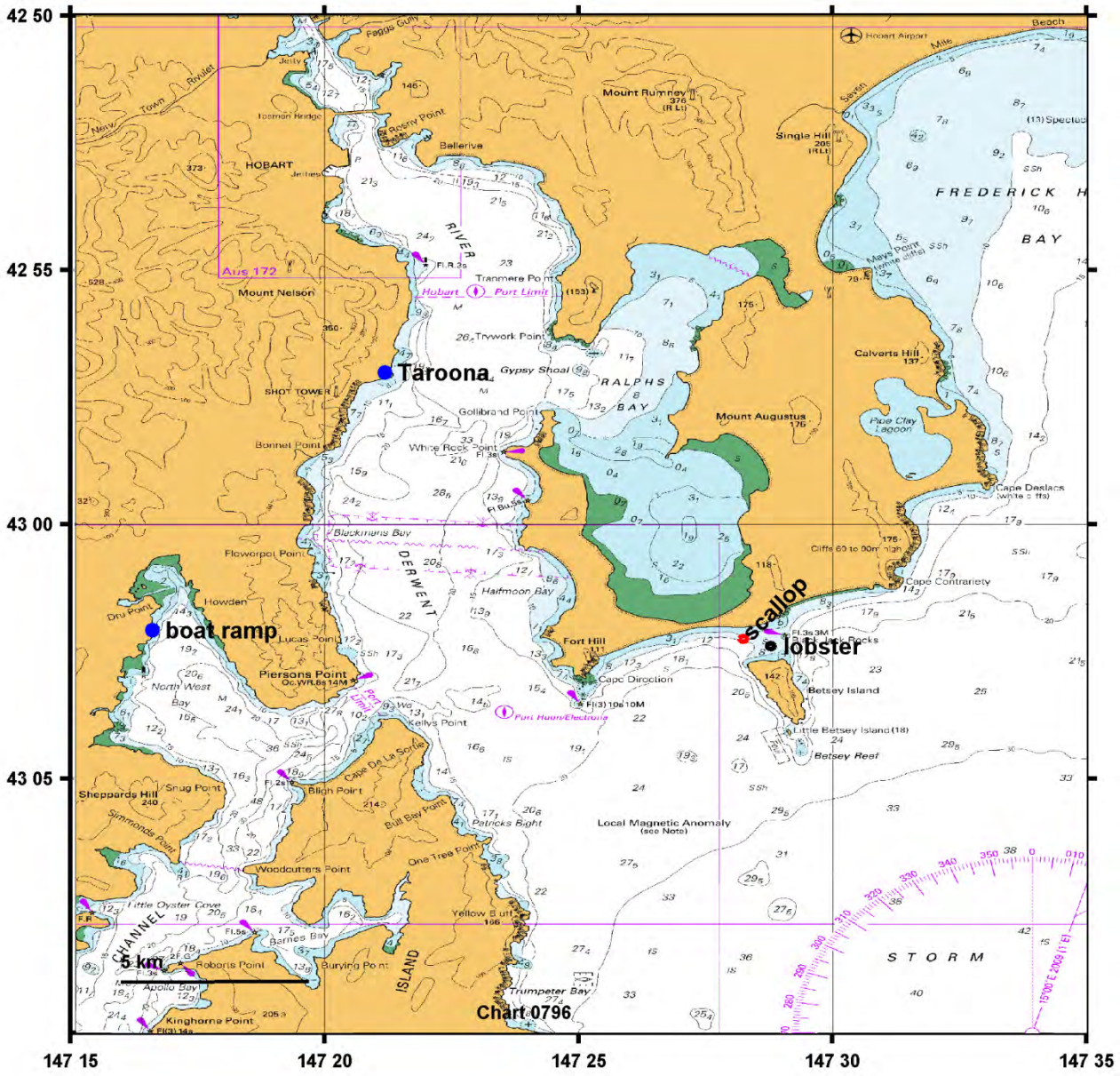


Figure 1. Location of scallop and lobster experimental sites inside Betsey Island, boat ramp and Taroona aquaculture facility were test animals were housed.

Table 1. Details of vessels, crew and equipment used during lobster and scallop experiments. Columns are: Experiment; vessel; crew on source vessel with MP = Mike Porteus, JS = Jayson Semmens, RM =Rob McCauley, RD = Ryan Day, QF = Quinn Fitzgibbon, GE = Graham Ewing (skipper), MF = Marion Fourquez (marine mammal observer), A = Andrew (skipper), AW =Andrea Walters (marine mammal observer); SN = Stefan Nixon; air gun used; air gun offsets to GPS aerial and depth of gun ports below sea surface (astern = 5.1 m + tow length); air gun pressure; distance of near field hydrophone to gun ports; compressors used and operating pressures; and the gas storage bottles used.

Experiment	Vessel	Crew	Air gun	Gun tow offsets / depth (m)	Gun pressure (psi)	NF to gun (m)	Compressors and operating pressures	Gas storage
07 & 08-May-2013 pilot experiment 20 in^3 gun	RV Morana	MP, JS, MF, RM, RD, QF	Bolt 600-B 20 in^3	13.6 m astern 4.5 m depth	2000	0.8	Bauer 0.19 m^3 / min (190 L / min)	2 × G HP air cylinders
27 & 28-Jun-2013 scallop & lobster experiments	RV Morana	GE, JS, MF, RM, RD, QF	Sercel G Gun II, 45 in^3	21.3 m astern 0.9 m to port 5.1 m depth	2000	0.5	Bauer 0.19 m^3 / min (190 L / min)	2 × G HP air cylinders
13 to 31-Jul-2014 scallop & lobster	FV ShelleTon	A, AW,RM, SN, QF	Sercel G Gun II, 150 in^3	27.7 m astern 0.85 m to port 5.1 m depth	1300 lob 1 2000 lob 2 2000 scallop	0.5	Bauer-70, 0.66 L /min & Munchen, 0.57 L / min in parallel, blow off 350 bar (5076 psi), storage pressure 300 bar (4351 psi)	2 × G HP air cylinders
Feb-2015 scallop & lobster	FV ShelleTon	A, AW, MP, RM	Sercel G Gun II, 150 in^3	24.4 m astern 0.85 m to port 5.1 m depth	All 2000	0.5	Bauer-70, 0.66 L /min & Munchen, 0.57 L / min in parallel, blow off 350 bar (5076 psi), storage pressure 300 bar (4351 psi)	4 × G HP air cylinders

Standard conditions used in experiments were:

1. Shot interval 11.6 s
2. near field hydrophone HTI U 90 (serial number 454042, sensitivity -199.1 db re V2/µPa2), cabled to 0 dB gain preamp, recorded by SD722 digital recorder (24 bit, 48 kHz sample rate-6 to 0 dB gain), hydrophone hard mounted on frame above gun, gun hanging from chains
3. *RV Morana*, 8 m alloy work boat, twin 150 HP outboards, gantry to deploy air gun. *FV ShelleTon*, 35' (11 m) length, beam 3.95 m, 10 tonnes gross, single screw, 400 HP Yanmar main engine, reasonable size back deck for working, small gantry and stern cradle to deploy air gun from. GPS location logged every 1 s (two Holux GPS units)

Airgun operations

A single air gun was used in all experiments, a Sercel G Gun II with a 45 in^3 chamber was used in 2013 and the same air gun with a150 in^3 chamber used in 2014 and 2015. Details of the experimental setup are listed in Table 1 and details of each run of the source vessel for control and active runs in all experiments are listed in Table 2.

For monitoring the vessel location two GPS units logging every 1 s were mounted side by side, inboard of the respective vessel, with the aerial and tow offsets used to calculate air gun location. The air gun was operated every 11.6 s at an approximate mean speed of 1.8 ms^{-1} or 3.6 knots. A near field hydrophone (HTIU-90) was located near the gun ports (see Table 1) and all near field air gun signals logged to a Sound Devices SD722 or SD744 digital recorder, using a 0 dB pre-amplifier and -6 dB gain on the recorder and 24 bit, 48 kHz sampling. The air gun was operated from a bank of G size high pressure air bottles (350 Bar or 35 MPa rated, two bottles in 2013, four bottles in 2014 and 2015). In 2013 a single Bauer 0.19 m^3min^{-1}compressor was used, while in 2014 and 2015 twin compressors were operated in parallel. The system and gas bottles were pumped to 300 Bar with all safety relief valves set at 350 Bar. For the 45 in^3 and 150 in^3 setups respectively, approximately 120 or 110 shots were available at full pressure (2000 psi, 13.8 MPa) with full gas bottles and the compressor/s running. The time taken to fill bottles was highly dependent on ambient temperature. An air gun firing control system which triggered a log file via TTL output on a firing pulse was used. The minimum vessel crew was four: skipper, marine mammal observer and two air gun operators. The air gun was deployed and recovered charged via a lifting davit.

Airgun signal measures

To monitor the air gun signal exposure received by target animals and the normal ambient noise regime at the site, three or four sea noise loggers were set on the seabed over the full experimental duration. All sea noise loggers recorded pressure while two recorded ground borne vibration (velocity) via geophones. The sea noise loggers were located next to lobster pots or scallop cages at each end of the pot or scallop lines during experiments. The sea noise loggers were Curtin University designed, CMST-DSTO sea noise recorders (www.cmst.curtin.edu.au/products). All noise loggers had pressure sensors fitted using High Tek HTI U90 or Massa TR1025C hydrophones. Two noise loggers were modified to include 3-axis geophone sensors to measure ground borne vibration (ION Geophysical, SM-6/U-B 10 Hz vertical and SM-6/H-B 10 Hz horizontal). Two geophones were aligned at opposing 45^o angles from the horizontal and one was aligned vertically. All sea noise logger housings were placed on the seabed by divers. The housings were stainless steel, 6 mm wall thickness and had plastic cross bars with weights at each end to stop the housing rolling. The weight of the housing, cross bar and batteries (~ 50 kg underwater) ensured the housing was firmly coupled to the seabed. The hydrophones were external to the housing and sat freely on the seabed with the hydrophone cable weighted to prevent it moving. All sea noise recorders were calibrated for the pressure response by inputting white noise of known level (traceable standard) into the logger with the white noise source and hydrophone in series. Analysis of the logged white noise signal gave the system gain with frequency, accounting correctly for the impedance match of the hydrophone, pre-amplifier and system electronics. Example calibration curves are shown on Figure 2 for the 2015 instruments (pressure). The system gain curve was used with the known hydrophone sensitivity to convert the logged volts to Pascals with the system response calibrated

from 1 Hz to the anti-aliasing filter frequency. The on-board noise logger clocks were set to GPS, UTC transmitted time before deployments using hardware and software and the drift read after recovery to give absolute timing accuracies of < 0.25 s. Total system gains for the pressure sensors were varied through -20, 0, 20 or 40 dB depending on where the instrument was being used while geophone gains used were 20 or 40 dB. All receivers were on a duty cycle, the geophone loggers recorded for 22 of 30 or 44 of 60 minutes, while the pressure only receivers recorded for 26 of 30 or 51 of 60 minutes. The configurations and locations of sea noise loggers used for all experiments are listed in Table 3.

Table 2. Details of air gun deployment during lobster and scallop experiments. Given are: nominal name; date; time of start, time of end with elapsed time (minutes); number of air gun shots where applicable; source used; and mean vessel speed. Times are EST (UTC+10 hours) for July experiments or EST+1 (UTC+11 hours) for February / March experiments.

Experiment		Date	Start time	End time (Duration in minutes)	Shots	Source (in^3 / psi)	Speed (ms^{-1}, kn)
Lobster-1	Con.	03-Jul-2013	09:56:21	10:14:10 (17.8)	0	control	1.69, 3.28
Lobster-1	Exp.	03-Jul-2013	11:36:05	12:00:23 (24.3)	126	45 / 2000	1.71, 3.32
Lobster-2	Con.	21-Jul-2014	11:22:50	11:52:50 (30)	0	control	2.01, 3.91
Lobster-2	Exp.	21-Jul-2014	13:35:37	13:52:51 (17.2)	112	150 / 1300	1.95, 3.80
Lobster-3	Con.	28-Jul-2014	11:17:51	11:45:44 (27.9)	0	control	1.92, 3.74
Lobster-3	Exp.	28-Jul-2014	12:37:23	13:00:39 (23.3)	110	150 / 2000	1.84, 3.57
Lobster-4	Con.	26-Feb-2015	10:32:09	10:44:08 (12.0)	0	control	2.02 / 3.92
Lobster-4	Exp.	26-Feb-2015	12:16:10	12:48:41 (32.5)	131	150 / 2000	2.00 / 3.89
Scallop-1	Con.	27-Jun-2013	11:35:00	12:00:00 (25.0)	0	control	*
Scallop-1 P1	Exp.	27-Jun-2013	12:41:22	13:13:36 (32.2)	167	45 / 2000	0.99 / 1.92
Scallop-1 P2	Exp.	28-Jun-2013	09:32:29	09:43:58 (11.5)	59	45 / 2000	1.81 / 3.51
Scallop-1 P3	Exp.	28-Jun-2013	10:40:05	10:54:26 (14.4)	72	45 / 2000	1.8 / 3.49
Scallop-1 P4	Exp.	28-Jun-2013	11:41:35	12:00:54 (19.3)	95	45 / 2000	1.71 / 3.33
Scallop-2	Con.	24-Jul-2014	10:34:56	10:51:20 (16.4)	0	control	2.00 / 3.88
Scallop-2 P1	Exp.	24-Jul-2014	11:56:20	12:25:22 (29)	128	150 / 2000	1.87 / 3.62
Scallop-2 P2	Exp.	25-Jul-2014	11:07:10	11:21:22 (14.2)	67	150 / 2000	1.96 / 3.8
Scallop-2 P3	Exp.	25-Jul-2014	12:24:49	12:34:18 (9.5)	51	150 / 2000	1.99 / 3.87
Scallop-2 P4	Exp.	25-Jul-2014	14:44:31	14:56:30 (12)	63	150 / 2000	1.98 / 3.86
Scallop-3	Con.	02-Mar-2015	15:26:19	15:39:55 (13.6)	0	control	2.08 / 4.05
Scallop-3 P1	Exp.	02-Mar-2015	16:27:31	16:37:42 (10.2)	54	150 / 2000	1.90 / 3.68
Scallop-3 P2	Exp.	03-Mar-2015	07:06:46	07:18:13 (11.4)	61	150 / 2000	2.00 / 3.89
Scallop-3 P3	Exp.	03-Mar-2015	14:15:46	14:27:35 (11.8)	62	150 / 2000	1.94 / 3.76
Scallop-3 P4	Exp.	03-Mar-2015	15:39:53	15:54:08 (14.3)	74	150 / 2000	1.93 / 3.75

Note: * - no GPS data that run

Table 3. Details of sea noise loggers set during lobster and scallop experiments. Given are the: set number; electronics and system gain in dB (E02 / E45 geophone loggers - pressure gain / geophone gain, others split pressure channel gains - dB - in form: pre-amp, Ch1-1, Ch-2); SL /I - sample length and interval (minutes); number channels sampled; experiment; sample start time; sample end time; hour offset from UTC; latitude; longitude; and valid samples while the instrument was in the water. All times are local time with column *UTC* + being the hours post UTC time used.

set	E.	SL / I (mins)	kHz	Ch.	experiment	Date time in	Date time out	UTC +	Latitude	Longitude	Samples
3222	E02, 20, 0	51 / 60	4	4	Scallop-1, 0, 1-4	27-Jun-2013 19:35	01-Jul-2013 18:35	10	43º2.365'	147º28.176'	48 - 143
3222	E02, 20, 0	51 / 60	4	4	Lobster-1, 0 & 1	03-Jul-2013 19:35	03-Jul-2013 21:35	10	43º 2.444	147º 29.05	191 - 193
3223	E45, 20 / 20	51 / 98.7	4	4	Scallop-1, 0, 1-4	27-Jun-2013 18:35	01-Jul-2013 18:35	10	43º 2.360'	147º28.178'	1 - 197
3223	E45, 20 / 20	51.2 / 60	4	4	Lobster-1, 0 & 1	03-Jul-2013 18:35	03-Jul-2013 21:35	10	43º 2.325	147º 29.06	191 - 194
3224	E22, 0 / 20	51 / 60	6	2	Scallop-1, 0, 1-4	27-Jun-2013 19:35	28-Jun-2013 12:00	10	43º 2.356'	147º28.179'	4 - 30
3224	E22, 0 / 20	51 / 60	6	2	Lobster-1, 0 & 1	03-Jul-2013 18:50	03-Jul-2013 21:50	10	43º 2.413	147º 29.041	32 - 35
3331	E01, 40	25.6 / 30	8	1	Beach, Lobster-2, 0	16-Jul-2014 15:30	21-Jul-2014 08:25	10	43º 2.024'	147º 28.654'	17 - 242
3331	E01, 40	25.6 / 30	8	1	Lobster-2,0 & 1	21-Jul-2014 10:30	21-Jul-2014 14:55	10	43º2.024'	147º 28.654'	247 - 255
3331	E01, 20/40	25.6 / 30	4	2	Scallop-2, 0, 1-4	24-Jul-2014 10:00	25-Jul-2014 15:55	10	43º 2.023'	147º 28.657'	295 - 354
3331	E01, 20/40	25.6 / 30	4	2	Lobster-3, 0 & 1	28-Jul-2014 10:30	28-Jul-2014 13:25	10	43º 2.033'	147º 28.651'	360 - 365
3332	E09, 0, 0/20	25.6 / 30	4	2	Lobster-2, 0 & 1	16-Jul-2014 12:00	21-Jul-2014 14:55	10	43º2.448'	147º 29.041'	10 - 255
3332	E09, 0, 0/20	25.6 / 30	4	2	Scallop-2, 0, 1-4	24-Jul-2014 10:15	25-Jul-2014 15:10	10	43º2.367'	147º 28.169'	295 - 352
3332	E09, 0, 0/20	25.6 / 30	4	2	Lobster-3, 1	28-Jul-2014 11:45	28-Jul-2014 13:10	10	43º2.397'	147º 29.033'	362 - 364
3333	E02, 20, 20	22.2 / 30	4	1	Lobster-2, 0 & 1	16-Jul-2014 10:45	21-Jul-2014 14:37	10	43º2.313'	147º 29.073'	7 - 254
3333	E02, 20, 20	22.2 / 30	4	1	Scallop-2, 1-4	24-Jul-2014 11:00	25-Jul-2014 14:52	10	43º2.329'	147º 28.172'	297 - 352
3333	E02, 20, 20	22.2 / 30	4	1	Lobster-3, 0 & 1	28-Jul-2014 11:00	28-Jul-2014 13:22	10	43º2.350'	147º 29.047'	361 - 365
3389	E02, 20, 20	44.4 / 60	4	4	Lobster-4, 0 & 1	26-Feb-2015 09:00	26-Feb-2015 12:00	11	43º2.523'	147º 28.318	1 - 4

		44.4 / 60	4	4	Scallop-3,0, 1 & 2	02-Mar-2015 15:00	03-Mar-2015 07:00	11	43º2.316'	147º 28.101'	17 - 33
		44.4 / 60	4	4	Scallop-3, 3 & 4	03-Mar-2015 13:00	03-Mar-2015 16:00	11	43º2.367'	147º 29.015'	39 - 42
3390	E45, 20, 20	44.4 / 60	4	4	Lobster-4, 0 & 1	26-Feb-2015 09:10	26-Feb-2015 12:10	11	43º 2.432	147º 29.095	1 -4
		44.4 / 60	4	4	Scallop-3,0, 1 & 2	02-Mar-2015 14:00	03-Mar-2015 07:00	11	43º 2.422	147º 27.441	16 - 33
		44.4 / 60	4	4	Scallop-3, 3 & 4	03-Mar-2015 14:00	03-Mar-2015 16:00	11	43º 2.439	147º 27.412	40 - 42
3390	E45, 20, 20	44.4 / 60	4	4	Lobster-4, 0 & 1	26-Feb-2015 09:10	26-Feb-2015 12:10	11	43º 2.432	147º 29.095	1 -4
		44.4 / 60	4	4	Scallop-3,0, 1 & 2	02-Mar-2015 14:00	03-Mar-2015 07:00	11	43º 2.422	147º 27.441	16 - 33
		44.4 / 60	4	4	Scallop-3, 3 & 4	03-Mar-2015 14:00	03-Mar-2015 16:00	11	43º 2.439	147º 27.412	40 - 42
3391	E28, 0, 0, 20	51.2 / 60	4	2	Lobster-4, 0 & 1	26-Feb-2015 10:00	26-Feb-2015 12:00	11	43º 2.448	147º 29.019	3 - 5
		51.2 / 60	4	2	Scallop-3,0, 1 & 2	02-Mar-2015 15:00	03-Mar-2015 07:00	11	43º 2.326	147º 28.194	17 - 33
		51.2 / 60	4	2	Scallop-3, 3 & 4	03-Mar-2015 14:00	03-Mar-2015 16:00	11	43º 2.326	147º 28.13	40 - 42
3392	E29, 0, 0, 20	51.2 / 60	4	2	Lobster-4, 0 & 1	26-Feb-2015 10:10	26-Feb-2015 12:10	11	43º2.329	147º 29.054	4 - 6
		51.2 / 60	4	2	Scallop-3,0, 1 & 2	02-Mar-2015 14:00	03-Mar-2015 07:00	11	43º 2.385	147º 28.157	20 - 37
		51.2 / 60	4	2	Scallop-3, 3 & 4	03-Mar-2015 14:00	03-Mar-2015 16:00	11	43º 2.415	147º 28.19	44 - 46
3432	E29, 20, 20	8.6 / 15	12	1	Taroona Reserve	30-Apr-2015 12:15	28-Jul-2015 16:00	10	42º 57.217'	147º 21.469'	227 - 8778
3433	E51, 20, 20	7.7 / 15	96	1	De Witt Island	16-Jun-2015 12:45	16-Jul-2015 01:03	10	43º35.23’	146º 22.41’	4733 - 7566

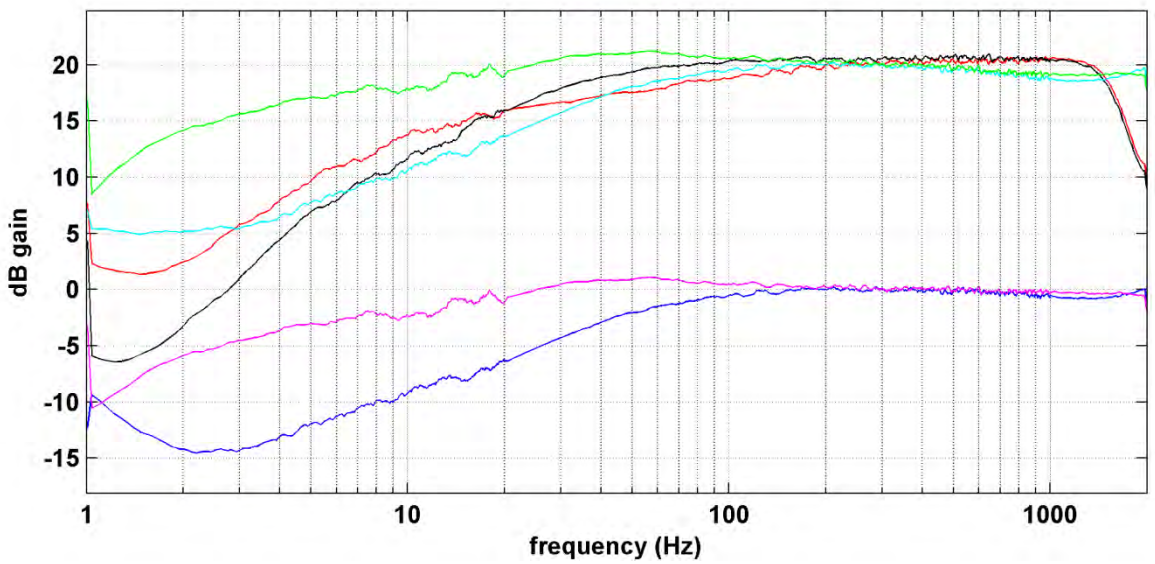


Figure 2. Sample sea noise logger system gain (pre-amp and recording system) showing frequency from the 2015 experiment: red - 3389, 20 dB system gain; black - 3300, 20 dB gain; blue - 3391, 0 dB gain; cyan - 3391, 20 dB gain; green - 3392, 20 dB gain; magenta - 3392, 0 dB gain. The curves were used over the frequency range above 5 Hz.

Temperature measures

Temperature loggers (Aquatech 520T) were located on the sea noise loggers to record seabed water temperature.

Air gun signal analysis and units

All times given here are Australian Eastern standard Time (EST = UTC + 10 hours) or EST with daylight saving (UTC + 11 hours). All times zones are indicated appropriately. All air gun and spatial analysis has been carried out in the Matlab (The Mathworks Inc.) environment using purpose built software. Air gun signals were analysed by:

1. In a graphical user interface (GUI) designed to deal with air gun signal extraction, visualising each high gain sea noise sample (spectrogram and waveform) with potential air gun signals, obtaining a voltage threshold which delineated air gun signals from the noise and setting the pre- and post- time brackets around the detections (the time window to analyse the signal in);
2. Using the signal filtered to keep the waterborne energy (removing ground borne energy), located the leading edge of each air gun signal using the voltage threshold set and a minimum time limit of how far apart consecutive signals must be (5 s used);
3. Checking the input voltage of each air gun signal for overloads and loading the low gain channel if an overload was found (the sea noise loggers are considered as overloaded if the voltage is > 2.4 V or < -2.4 V).
4. Displaying the location of the waterborne arrival in the GUI and manually removing any false detections.

5. Obtaining a time period to bracket the waterborne signal with. This was nominally set at ± 3 s but altered if the air gun signal had leading or trailing energy which fell outside of this window.
6. Bracketing the identified air gun signal time window using a multiple of two points, extract the signal and calibrate to Pa in the time domain accounting for variation in the system gain with frequency and using the system hydrophone sensitivity for the appropriate channel (see below). This step gave the calibrated air gun signal waveform in Pa.
7. Calculating a suite of signal descriptors as described in McCauley *et al.* (2003) plus identifying the time of air gun signal waterborne arrival.
8. Calculating the power spectra of each sample (as close as possible to a 1 Hz bandwidth used).
9. Looping through all identified air gun signals and saving the signal descriptors, received times, power spectra and the calibrated air gun waveform.

Each air gun signal was calibrated from volts to Pa by extracting the noise logger signal bracketing the identified air gun signal in a multiple of two points which was greater than the identified signal length and passing this to a program which:

- Returned the FFT of the input waveform section (multiple of two points) at a frequency resolution of close to 0.1 Hz.
- Calculated the system gain in linear units at a frequency spacing the same as given by the above FFT step, from 0 Hz to the Nyquist frequency, this gain including the hydrophone sensitivity.
- Applied the linear gain correction to the FFT amplitude.
- Assumed a unity phase correction applied to the FFT phase.
- Inverted the FFT back into the time domain.
- Extracted the required air gun signal (since it was of shorter duration than the section calibrated) from this corrected section of the waveform.

The analysis gave the air gun signal descriptors, power spectra, received arrival time of signals (logger clock corrected for drift) and the signal waveform. The time of each received air gun signal was then used to extract spatial information of the receiver location relative to the source. This involved:

1. Using the received shot time at the sea noise logger (waterborne arrival) to locate the closest shot in the air gun track file, then iterating the time / range of this and the previous few shots allowing for signal travel time, to find the fired shot point which best matched the fired time plus estimated travel time to give the sea noise logger received time;
2. For the identified air gun fired location, calculating: the receiver range (horizontal and slant range); the air gun speed and heading; the take-off angle of the receiver to the air gun heading (i.e. angle of the receiver from the air gun heading);
3. Save all data.

All data was saved in a standard format. Of the 16 signal parameters saved peak pressures, *rms* pressure, sound exposure level and signal duration were pertinent here. The signal duration was defined as the time taken for 90% of the signal energy to pass with the time at which 5% and 95%

of the signal energy reached defining the air gun signal onset and end, as per McCauley *et al.* (2003).

The geophone data was extracted from the noise loggers using the time bounds defined for the appropriate noise logger air gun waterborne signal. A linear calibration was assumed and applied to correct the saved volts to velocity (ms^{-1}), according to the system gain used (0 or 20 dB) and the specifications of the particular geophone. The geophone response had been checked and found to match the manufacturer's specifications. The geophones gave two horizontal (90^o to each other) and one vertical velocity. The respective velocities were differentiated to give acceleration (ms^{-2}) in the vertical or the vector sum in the horizontal. For analysis here the absolute magnitude of the three component acceleration vector has been used, and termed ground roll acceleration. This was calculated using a Matlab vector function (cart2pol.m). The acceleration magnitude has been expressed throughout in linear terms (ms^{-2}) or to show trends with range, in decibel terms relative to 1 ms^{-2} (i.e. dB re $1ms^{-2}$ as $10*\log_{10}(\text{LinearValue}/1)$).

In order to compare exposure histories for sound exposure levels and ground acceleration between the experiments conducted here and pass-bys of a commercial seismic survey a scenario of an actual 3D seismic survey was configured. Fitted curves for SEL with range and ground motion form commercial seismic vessels were used to give estimates of sound exposures with the seismic source vessel passing by at some nearest range. Full details are given in the results, in order to keep the rationale and results complete.

Lobster Methods

Animal care and experimental design

Southern rock lobsters (*Jasus edwardsii*) used in this study were held at the Institute for Marine and Antarctic Studies, Taroona, Tasmania (IMAS) four 3400 litre (2 m × 2 m × 0.85 m) tanks supplied with 50 µm filtered flow through seawater (1 exchange per hour) at ambient temperature (*ca.* 13°C in 2013 and 2014 experiments, *ca.* 17°C in 2015 experiment) and provided with aeration. On receiving lobsters from the wild they were weighed, measured for carapace length, observed for injury, disease or limb damage and tagged with numbered and colour coded cable ties before random assignment of treatment (control/exposed) and sampling point groups and random distribution among the 4 tanks with control and exposed lobsters cohabitating. Lobsters were held for five days prior to deployment in lobster pots at the field site, where they were allowed two days acclimation prior to the experiment. Following the field experiment, lobsters were returned to IMAS and held in the same tank. During all holding periods, lobsters were fed live blue mussels (*Mytilus galloprovincialis*) *ad libitum* twice weekly, filters cleaned daily and tanks cleaned biweekly. During daily tank maintenance the lobsters were visually assessed for any signs of moribundity or mortality through observation of behavioural responses and movement (Stoner 2012).

On the day of the experiment, lobsters were transported in plastic crates (Viscount Plastics IH954, 735mm × 535 mm × 305 mm) and placed into a fiberglass, trailer mounted tank filled with seawater for transport. The seawater was constantly aerated with O_2 to maintain a saturation of 100%, which was monitored using a dissolved oxygen probe. Lobsters were moved into a large bin (1.2 m × 0.75 m × 1 m) of seawater on the vessel for transport to the field site. Again, the seawater was aerated using O_2 to maintain a saturation of 100%, which was monitored using a dissolved oxygen probe.

At the field site, lobsters were randomly assigned to lobster pots, which were Tasmanian commercial fishing industry standard, constructed with a steel frame (750mm × 750 mm × 400 mm) and covered with a 1cm net mesh. The pots were modified through removal of the steel mesh bottom, which was replaced with a net mesh to conform to and allow contact with the reef substrate, and the replacement of the top mesh of the pot with a Perspex sheet to allow video recordings to be made during the experiment.

Pots were lowered by hand into the water until they rested on the reef structure and marked with surface buoys. Pot orientation and contact with substrate was confirmed by divers immediately prior to exposure experiments. In 2013 and the first experiment of 2014, 20 lobster pots were set over a transect that ran approximately 250 m north-south on the reef substrate at the eastern end of the channel separating Betsy Island and Blackjack Reef. In the second experiment of 2014 and in the 2015 experiment, the pots were set over a transect that ran approximately 100 m at the same location. Dan-buoys marked the end of the lines of pots with each pot having a small surface buoy. Noise loggers were located at the end of the lobster pot lines and in the centre of the line. For the second experiment in 2014 only 10 pots were deployed. Locations of the control and exposed runs for all lines and the pot locations in 2013 and 2014 are shown on Figure 3 and for the 2015 experiment on Figure 4.

The general experimental regime (see details of each experiment below for any specific variations) consisted first of an acclimation period of two days following deployment. Following this period, the experimental regime began with the positioning of the seismic vessel approximately 1 km to the west of the north-south line of lobster pots. The air gun vessel conducted a control run, in which the air gun was charged and deployed but not fired. The run consisted of the vessel approaching the centre of the transect line at a mean speed of approximately 1.8 ms^{-1} before circling the pot transect to ensure the air gun passed by all pots in close proximity. The same vessel path was used in subsequent exposure runs. Following the control run, the pots randomly assigned to the control group (50% of all pots) were sequentially retrieved to the surface and the lobsters taken out and visually assessed for mortality and moribundity based on leg and antennae movements (Stoner 2012). For the day 0 sample period lobsters, reflex behaviour tests (details below) were performed immediately as the lobster was removed from the pot. All lobsters were then placed into the plastic storage crates and held in the bin of aerated seawater.

Following retrieval of all control pots, the exposure run was conducted in the same manner as the control run but with the air gun firing every 11.6 s. The retrieval process was repeated for the exposed treatment pots. At the conclusion of the retrieval process, the vessel returned to the shore, where the lobster holding crates were placed back into the trailer mounted tank and transported back to IMAS. On return to IMAS, day 0 lobsters were sampled immediately following the procedures detailed below. Lobsters from all other treatments were removed from the crates and placed into the four holding tanks according to previously determined random assignment, with controls and exposed lobsters cohabitating. These lobsters were later sampled depending on the predetermined sampling protocol.

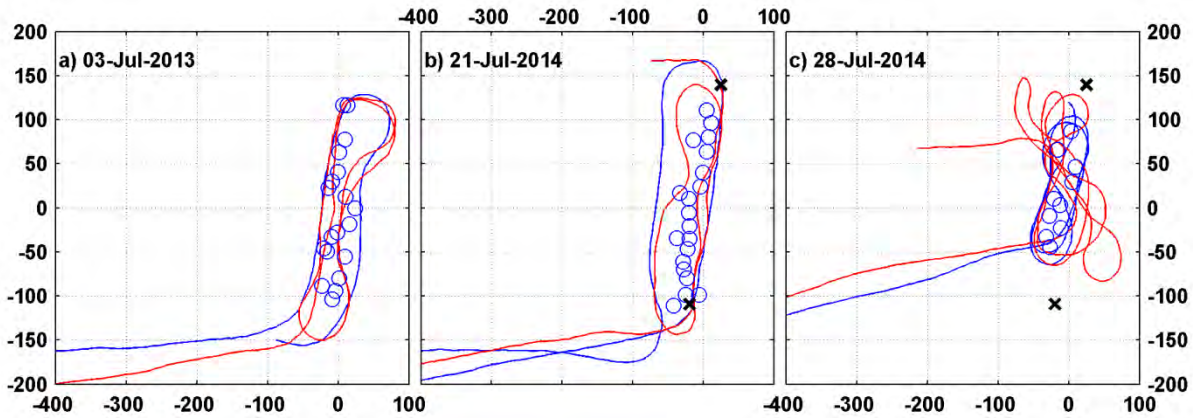


Figure 3. Control (blue) and air gun runs (red) during the three lobster experiments 2013-2014. The circles are pot locations (a: winter 2013 45 in^3 standard pressure experiment n=20, b: winter 2014 150 in^3 low pressure experiment n=20, c: winter 2014 150 in^3 standard pressure experiment n=10) and the crosses sea noise logger locations. The grid scale is in m which has been arbitrarily zeroed to the approximate centre of the line of pots. The 2015 experiment configuration was similar to the 28-Jul-2014 (panel c) configuration.

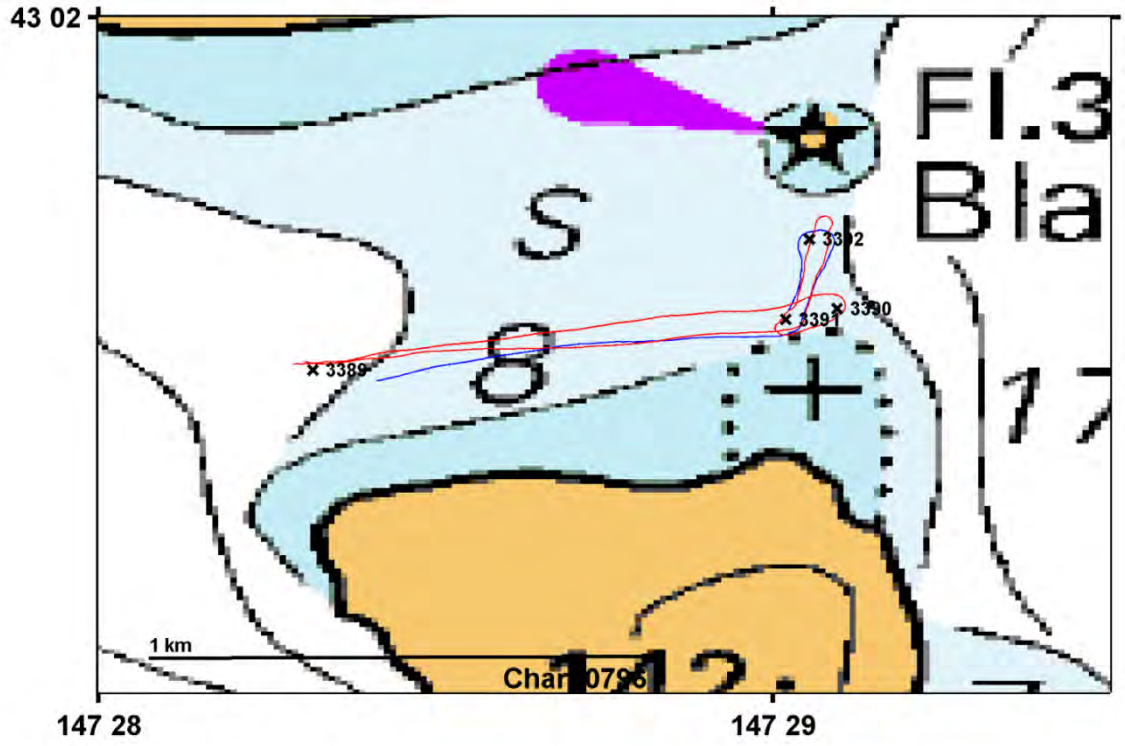


Figure 4. The 2015 lobster experimental passes and sea noise logger locations. The blue line is the vessel GPS track during the control run, the red line the active air gun vessel track (the track goes beyond the end of air gun operations).

Four experiments examining the effects of seismic exposure on lobster were conducted in this study:

Winter 2013, 45 in^3 standard pressure experiment

Field work for the first experiment was conducted over 1-3 July 2013, in which berried female lobsters were exposed to signals from a 45 in^3 air gun operated at 2000 psi, referred to as the winter 2013, 45 in^3 standard pressure experiment henceforth. Egg extrusion occurs between May and June in *J. edwardsii* in Tasmanian waters (Smith *et al.* 2003a), so at the time of collection, eggs were estimated to be 4-6 weeks post-extrusion and had not yet developed an eye spot.

A total of 103 berried female lobsters with a mean carapace length (CL) of 96.0 ± 1.5 mm were obtained from commercial fishermen from several sites around Shoemaker Point, Tasmania (43° 35' 38.23" S, 146° 38' 03.69" E; Fig. 5). To determine whether time played a factor in any observed responses, 20 lobsters were randomly assigned into each of five sampling times: day 0, day 2 post-exposure, day 14 post-exposure, day 120 post-exposure, and a post-hatch group used to assess embryonic development following exposure. Each sample group was identified using colour coded antenna tags.

When lobsters were placed into pots (n=20) at the field site, one lobster from each of the five sample groups was placed into each pot, which represented an experimental replicate. Control and exposure treatment groups of lobsters were placed into identical but different pots (10 control and 10 exposure group pots). One or two additional lobsters over the required number for each replicate were randomly distributed amongst the lobster pots in case there was any loss due to escape, predation or mortality. Lobsters were left for a two day acclimation period before the control and exposure air gun runs were conducted.

On each sample day, the lobsters to be sampled, as identified by colour coded antenna tags, were collected from the four holding tanks and held in a plastic crate immersed in one of the holding tanks. Lobsters were haphazardly selected from this crate for sampling until all were done. The sampling procedure (details below) was, in order: tail tonicity reflex, righting, total haemocyte counts, haemolymph biochemistry; and then, following euthanasia: carcass mass, statocyst dissection, bundle mass, hepatopancreas mass.

Final sample sizes for each component of the experiment are given in Appendix I.

Winter 2014, 150 in^3 low pressure experiment

Field work for the second experiment was conducted over 14-21 July 2013, in which berried female lobsters were exposed to signals from a 150 in^3 air gun operated at 1300 psi, referred to as the winter 2014, 150 in^3 low pressure experiment henceforth. Similar to the first experiment eggs were in the early stage development and were estimated to be 6-10 weeks post-extrusion and had not yet developed an eye spot.

A total of 105 berried female lobsters with a mean carapace length (CL) of 91.2 ± 0.7 mm were obtained from commercial fishermen from approximately the same fishery sites (Shoemaker Point) as in the 2013 experiment (Fig. 5). Sampling groups were similar to those of the previous winter 2013, 45 in^3 standard pressure experiment with one exception: the lobsters used for the assessment

of embryonic development in the present experiment were retained until day 365 post-exposure to determine whether exposure resulted in any disruption of the moulting process.

Lobsters were assigned into treatment groups and sample times as in the previous experiment, and again, additional lobsters over the required number for each replicate were randomly distributed amongst the lobster pots in case there was any loss due to escape, predation or mortality. The only departure from the field methods of the previous experiment was that lobsters in this experiment had an acclimation period of 7 days due to technical difficulties with the air gun. This extended period led to some loss due to predation by sharks or seals, resulting in the differences in sample size for the sample points. All other aspects of the experiment were conducted as in the previous experiment.

Final sample sizes for each component of the experiment are given in Appendix I.

Winter 2014, 150 in^3 standard pressure experiment

Over 26-28 July 2014, 63 lobsters were obtained from the Crayfish Point Scientific Reserve (42° 57' 10.63" S, 147° 21' 17.42" E; Fig. 5) and exposed to signals from a 150 in^3 air gun operated at 2000 psi, henceforth referred to as the winter 2014, 150 in^3 standard pressure experiment. These lobsters were divided into the following three groups: 1) 21 (control n=11, exposed n=10) male lobsters ranging in size from 93-146 mm CL with a mean CL of 119.0 ± 3.0 mm, 2) 21 (control n=11, exposed n=10) berried female lobsters ranging in CL from 88-125 mm with a mean CL of 105.0 ± 1.7 mm estimated to be 5-7 weeks post-extrusion, and 3) 21 (control n=11, exposed n=10) berried female lobsters ranging in CL from 87-120 mm with a mean CL of 104.0 mm ± 2.0 mm also estimated to be 6-10 weeks post-extrusion.

Lobsters were randomly assigned to sample times and exposures as previously, however, the sample schedule was altered to accommodate for the lower number of animals. Male lobsters were sampled for tail tonicity and righting reflexes reflex behaviours on days 0, 2 and 14; for total haemocyte counts and haemolymph biochemistry on days 2 and 14; and were euthanized to collect carcass mass, statocysts and hepatopancreas mass on day 14. Berried female lobsters were sampled on the same schedule, with the same data collected and will be referred to as berried females sampled at days 0-14 henceforth. Berried female lobsters retained for assessment of embryo development and the quantity and quality of subsequent larvae, after which they were sampled at day 120 as per the winter 2014, 150 in^3 low pressure experiment. These lobsters will be referred to as berried females sampled at day 120 henceforth.

In addition to the altered sampling schedule, only 10 lobster pots were used for this experiment, with one lobster each from the males, berried females sampled at days 0-14 and berried females sampled at day 120 placed into each pot for the experiment, with additional lobsters over the required number for each replicate were randomly distributed amongst the lobster pots in case there was any loss due to escape, predation or mortality. All other aspects of field work were conducted as in the winter 2013, 45 in^3 standard pressure experiment.

Final sample sizes for each component of the experiment are given in Appendix I.

Summer 2015, 150 in^3 standard pressure experiment

To investigate the effects of exposure in warmer, summer conditions, an experiment was conducted over 24-26 of February 2015. In this experiment 54 female lobsters ranging from 84-98 mm CL with a mean CL of 90.8 ± 0.4 mm were obtained from commercial fishermen from the same fishery sites as the winter 2013, 45 in^3 standard pressure and winter 2014, 150 in^3 low pressure experiments (Shoemaker Point). As this experiment was conducted in summer, female lobsters were not berried. The 150 in^3 air gun configuration was used and was operated at 2000 psi, and the experiment is henceforth referred to as the summer 2015, 150 in^3 standard pressure experiment.

In this experiment, lobsters were randomly assigned to control and exposed treatments as before, and were sampled at two points: day 2 and day 14. Apart from the reduction in sample points and the lack of a reproduction component, the experiment was carried out as per the other experiments.

Final sample sizes for each component of the experiment are given in Appendix I.

Figure 5. Lobster experiment locations. 1) IMAS, site of lobster holding laboratory work for all experiments and the Crayfish Point Scientific Reserve where lobsters were collected for winter 2014, 150 in^3 standard pressure experiment; 2) Blackjack Rocks, field site; 3) Shoemaker point, lobster collection site for winter 2013, 45 in^3 standard pressure, winter 2014, 150 in^3 low pressure and summer 2015, 150 in^3 standard pressure experiments.

Sampling

Reflex behaviour

Two reflex behaviours were assessed in lobsters. The first test conducted was a quantification of tail tonicity, evaluated by measurement of the exposed portion of the first abdominal shell segment. To do so, lobsters were gently held by the carapace leaving the tail unsupported, and Vernier callipers were used to measure the amount of exposed shell to the nearest 0.1 mm. To ensure consistency across measurements, the same researcher conducted this measurement for every individual in every experiment. Measurements were standardised to carapace length and are referred to as relative tail gape throughout.

The second reflex test conducted was righting response, which was the time taken for lobsters to return to a dorsum-up position after being placed ventrum-up in a bin of seawater . For this assessment, “righted” was defined as returning to a position in which walking legs from both sides of the body were in contact with the bottom of the bin. Again, the same researcher conducted this assessment for every individual in every experiment to ensure consistency.

Statocyst analysis

After euthanasia and as a part of dissection, the statocysts of each lobster were removed and prepared for scanning electron microscopy following the standard techniques described by Felgenhauer (1987). Briefly, the basal segment of the antennules from each lobster were isolated and placed into 2.5% glutaraldehyde for 48 h, then dehydrated in a series of increasing ethanol concentrations. A rotary tool (Dremel) fitted with a ball tipped cutting bit was used to cut through the antennule cuticle and expose the statocyst. The statocyst was carefully split open using a scalpel and the statoconia was gently rinsed away with a jet of 70% ethanol from a transfer pipette. Following dissection, the opened statocyst was dehydrated to 100% ethanol, critical point dried, mounted on an aluminium stub and sputter coated.

Images taken using a scanning electron microscope were analysed for damage to hair cells using ImageJ 1.48v (Schneider *et al.* 2012). To conduct counts of all visible damaged and undamaged hairs within the statocyst, grid was overlaid on the image (Fig. 6) and the native ImageJ cell counter function was used. The overlaid grid was 0.01 mm^2, a dimension that was chosen arbitrarily to reflect a reasonable area from which to exclude damage to hairs as a result of mechanical damage resultant from dissection. Hair cells were considered damaged when lacking a hair, leaving only a pore. To conservatively account for damage incurred during dissection, any damaged cells occurring within the same grid square as physical damage to the statocyst capsule itself (e.g. where the capsule was split, any cracks or punctures, etc.) were excluded from counting, whereas healthy cells occurring near dissection damage were counted.

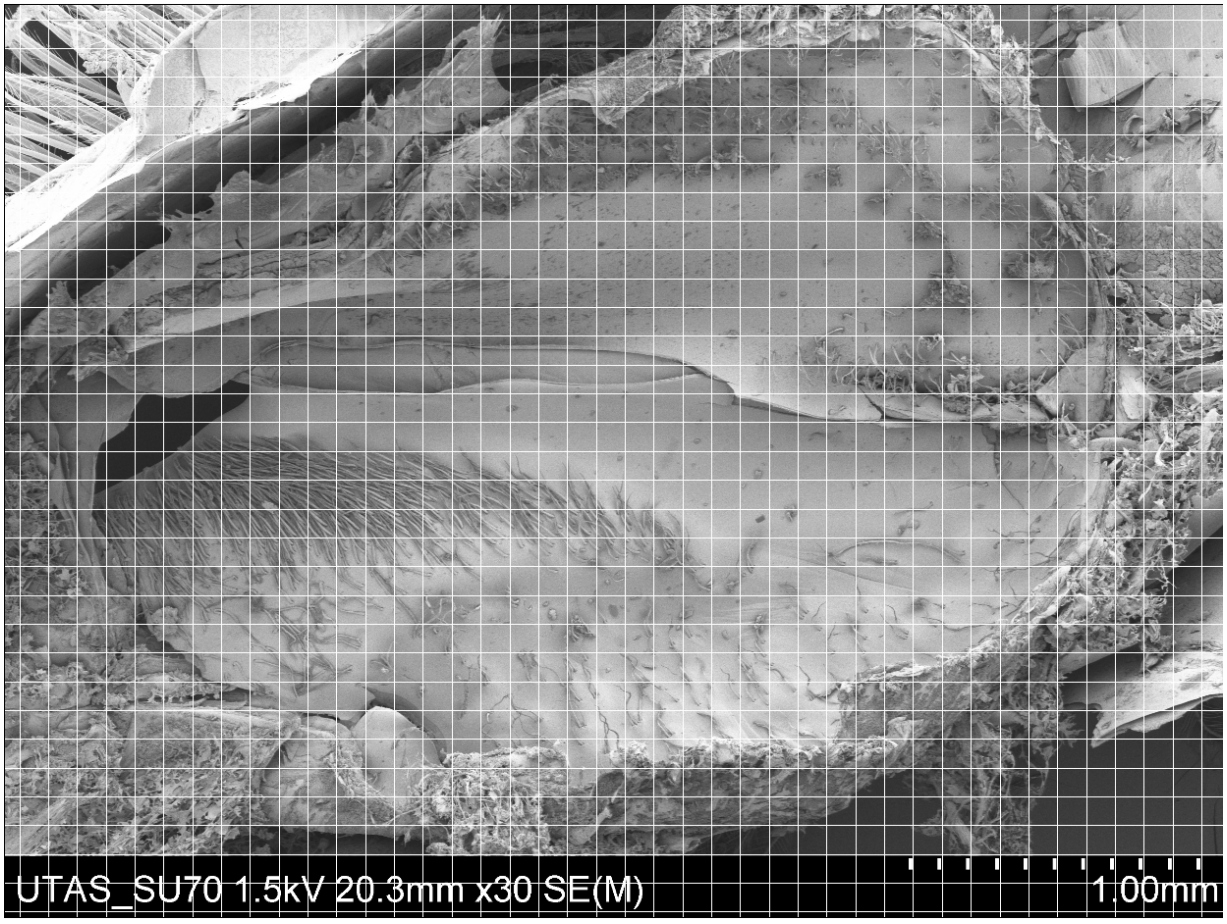


Figure 6. Lobster statocyst image analysis methods. Images of statocysts obtained using scanning electron microscopy were analysed using a 0.01 mm^2 grid imposed on the image and all visible damaged and undamaged hair cells were counted, with damage defined as hair cells lacking a hair, leaving only a pore. Damaged hair cells occurring within the same box as structural damage to the statocyst capsule were excluded from counting, whereas healthy hairs were included.

Haemolymph parameters

Following the reflex tests, haemolymph samples were taken from each lobster. Using a pre-chilled syringe to retard clotting, 5 ml of haemolymph was drawn from the sinus at the base of the rear walking legs. A 500 ul aliquot was immediately used to measure pH (Testo Instruments 405 pH probe) and refractive index using a digital refractometer (Hanna Instruments HI 96801). The refractometer was zeroed using deionized water as described by Simon *et al.* 2015. The measured Brix index can be converted to specific gravity (SG) with a conversion factor of SG=0.0041 x Brix + 1.0. Two 1000 µl aliquots, , were centrifuged at 3,000 × g for 4 min, after which the haemocyte-free supernatant was removed, pipetted into a cryovial and snap frozen in liquid nitrogen for later biochemical analysis. A final 500 µl aliquot was added to 500 µl of anticoagulant (Baker's formol calcium; 2% NaCl, 1% calcium acetate, 4% formaldehyde) for use in total haemocyte counts (THC).

The 1000 µl centrifuged supernatant sample was shipped, frozen on dry ice, to Diagnostic Services at the Atlantic Veterinary College, University of Prince Edward Island, Canada, and analysed using a Cobas c501 automated biochemistry analyser (Roche Diagnostics Corporation, Indianapolis, IN, USA) for a full blood profile consisting of the electrolytes (mmol l-1 = mM) sodium (Na), chloride (Cl), potassium (K), magnesium (Mg) and bicarbonate (bicarb); minerals (mmol l-1) calcium (Ca) and phosphorus (P); metabolites (mmol l-1 = mM) glucose (Gluc), lactate (Lact), cholesterol (Chol), triglyceride (Trig), total protein (TP, in g l-1), urea, and uric acid (Uric, in µmol l-1); enzymes (U l-1) lipase (LIP), amylase (AMY), alanine (ALT) and asparate (AST) aminotransferases, alkaline phosphatase (ALP), sorbital (SDH) and glutamate (GDH) dehydrogenases, and gamma-glutamyl transferase (GGT).

The 1:1 (v/v) mixture of haemolymph and anticoagulant was used for total haemocyte counts using an improved Neubauer haemocytometer under 40x magnification, with digital photographs taken of the slide which were later analysed using the built in cell-counter in NIH ImageJ v. 1.48 (Schneider *et al.* 2012).

Condition

Following haemolymph collection, lobsters were euthanized via immersion in an ice-seawater slurry. Mass and carapace length were measured and egg bundles were removed from berried females and weighed. Bundle mass was used to calculate bundle index (BI) for all berried animals. The hepatopancreas was dissected out and weighed and used to calculate hepatopancreas index (HPI= [hepatopacreas mass · body mass^{-1}] × 100; Simon *et al*. 2015). In cases of berried lobsters, the egg bundle mass subtracted from the body mass prior to calculation of HPI.

Embryonic development, larval quantity and quality

Hatching

In the winter 2013, 45 in^3 standard pressure, winter 2014, 150 in^3 low pressure and winter 2014, 150 in^3 standard pressure experiments, assessment of the impact of seismic exposure on the development of the embryos was assessed. Control and exposed lobsters from the post-hatch sample group were maintained in tanks at IMAS and held in the same conditions as prior to the experiment until the hatch of larvae. In the 2013, 45 in^3 standard pressure experiment, hatching began in mid-September (3 months after the field work was conducted) and was complete in mid-October (4

months after the field work was conducted). In the 2014 experiments, hatching began at the end of September (3 months after the field work was conducted) and continued until early November (4 months after the field work was conducted). Just prior to larval hatching, as determined by eye index aging (Tong *et al.* 2000), lobsters were moved from communal housing to 20 L isolation tanks with 300 mm × 150 mm panels of 100 µm mesh (Smith *et al.* 2003b) to allow for collection of larvae from each individual. Each isolation tank received flow of filtered seawater at ambient temperatures. Isolation tanks were checked daily for hatches, which were drained into a graduated 20 L vessel for subsequent analysis.

Fecundity

Counts of hatched larvae were performed for each individual on every day hatched larvae were present. To count larvae, the volumetric estimate described by Smith and Ritar (2005) was used. Briefly, larvae were placed into a known volume of water (10, 15 or 20 L, depending on visual estimation of larval density). Larvae were suspended via thorough mixing of the water to ensure an even distribution. Water samples (n=5) of volumes inversely proportional to larval density (50, 125 or 250 ml) were taken so that at least 30 larvae were counted in each sub-sample. The larvae contained in each sample counted while the sample was decanted into a beaker. The mean number of larvae from the 5 samples was averaged to provide a hatch count.

Morphometrics

On the first day of an observed hatch, around 40 larvae from each individual were collected and placed between two petri dishes which were then gently pressed together to displace excess water and keep larvae prostrate and planar, allowing for measurements to be made using a projection microscope at 20X magnification. From each sample 20 larvae were measured for length and width to the nearest mm (± 0.5 mm). Any larvae that were not lying prostrate were not measured, as a prostrate posture was necessary for accuracy. Any naupliosoma that had not yet metamorphosed into larvae were not measured, as the naupliosoma stage is a transient, pre-larval stage that lasts for 30 minutes or less (MacDiarmid 1985) and has a curled or folded posture that prevents accurate measurement. During the measurement process, larvae were observed for any apparent morphological abnormality. Larval morphological measurement confirmed that all observations were conducted during the first instar phyllosoma stage as described by Lesser (1978).

Calorimetry

On the third day of hatching, 120 larvae from each hatch were counted, collected into 5 ml sample tubes and snap frozen using liquid nitrogen. Tubes were stored in either liquid nitrogen or a -80° freezer until they were freeze dried. Following the freeze drying process, tubes were sealed and stored in a -80° freezer. To measure the energy content of each sample ($MJ \cdot kg^{-1}$), the freeze dried larvae were weighed to the nearest 0.01 mg and measured for energy using a microcalorimeter according to the manufacturer instructions.

Activity

On the second day of each hatch, larvae were tested for survival using an elevated temperature + reduced salinity activity test, which has been shown to be an effective measure of competency of larvae by Smith *et al.* (2003). Briefly, 20 larvae from each hatch were placed into 200 ml plastic

sample jars containing 10‰ seawater held at 21°C using a heated water bath. Larvae were observed at 3 min intervals and the number of larvae prostrated on the bottom was recorded until no larvae remained active. The number of prostrate larvae within each time interval was averaged for the 3 replicates and used for Kaplan-Maier survival analysis.

Statistics

Reflex behaviours

Data for relative tail gape from the winter 2013 45 in^3, winter 2014 150 in^3 low pressure and summer 2015 150 in^3 standard pressure experiments were each tested for normality using Shapiro-Wilks tests and equality of variances using Levene's tests. All three data sets met ANOVA assumptions, so were analysed using two-way ANOVA with treatment and sample time as factors and α=0.05, followed by post hoc Tukey HSD tests for any significant results. For the winter 2014 150 in^3 standard pressure experiment, relative tail gape from males and berried females sampled at days 0-14 was sampled at days 0, 2 and 14, so values were first checked for equality of variances using Levene's test, then the means were compared within each group using mixed-design ANOVAs with sample time as the within-subject factor and treatment as the between-subject factor. Mauchley's test was used to test the assumption of sphericity and group 1 failed to meet this assumption, so a Greenhouse-Geisser correction was applied. For berried females sampled at day 120, Welch two-sample t-tests were used for comparison.

Righting data from the winter 2013 45 in^3 standard pressure experiment failed the test for normality, so were transformed following a Box-Cox analysis which indicated λ = -0.02. Following transformation, ANOVA assumptions were met and data were analysed using a two-way ANOVA with exposure and time as factors, followed by post hoc Tukey HSD tests for any significant results. Righting data from the winter 2014 150 in^3 low pressure experiment also failed the test for normality, which was corrected with a square root transformation, after which the data were analysed using a two-way ANOVA with exposure and time as factors, followed by post hoc Tukey HSD tests for any significant results. Summer 2015 150 in^3 standard pressure experiment, data were log transformed to fit ANOVA assumptions and then analysed using a two-way ANOVA with exposure and time as factors, followed by post hoc Tukey HSD tests for any significant results. For the winter 2014 150 in^3 standard pressure experiment, relative tail gape from males and berried females sampled at days 0-14 was sampled at days 0, 2 and 14, so values were first checked for equality of variances using Levene's test, then means were compared within each group using mixed-design ANOVAs with sample time as the within-subject factor and treatment as the between-subject factor. Mauchley's test was used to test the assumption of sphericity and both males and berried females sampled at days 0-14 failed to meet this assumption, so a Greenhouse-Geisser correction was applied. For berried females sampled at day 120, Welch two-sample t-tests were used for comparison.

Statocyst analysis

To analyse statocyst hair cell damage, the proportion of damage in statocysts from control lobsters was compared first using a logistic regression model with a binary outcome of healthy versus damaged for each individual hair, with carapace length, mass, sample time, zone of the statocyst,

and exposure level considered as factors. Next, control treatments were compared to exposed treatments from the respective experiments using a similar binomial regression.

Righting time analysis

A generalised linear model was used to examine the relationship between exposure and righting times. Righting time data were log transformed as supported by a Box-Cox analysis and the proportion of damage, carapace length, treatment and experiment were used as factors.

Haemolymph parameters

Haemolymph pH and refractive index data from the winter 2013 45 in^3 standard pressure, winter 2014 150 in^3 low pressure and summer 2015 150 in^3 standard pressure experiments all failed to meet ANOVA assumptions, so these data were analysed using two-way randomised permutation ANOVAs with exposure and sample time as factors, using 5000 iterations following Manly (2007), followed by post hoc Tukey HSD tests for any significant results. For the winter 2014 150 in^3 standard pressure experiment, haemolymph pH and refractive index from males and berried females sampled at days 0-14 were sampled serially at days 2 and 14, so values were first checked for equality of variances using Levene's test, then means were compared within each group using mixed-design ANOVAs with sample time as the within-subject factor and treatment as the between-subject factor. For berried females sampled at day 120, Welch two-sample t-tests were used for.

For the winter 2014, 150 in^3 low pressure experiment the effect of exposure and sample time was tested using a two-way ANOVA, followed by post hoc Tukey HSD tests for any significant results. For the winter 2014, 150 in^3 standard pressure experiment, the effect of exposure and sex (male vs female) at day 2 post-exposure, on the haemolymph biochemistry of lobsters was tested by a two-way ANOVA. At day 120, only female lobsters were sampled and the effect of exposure and time post-exposure (day 2 vs day 120), as well as their potential interaction, on the haemolymph biochemistry of lobsters was tested by a two-way ANOVA. Before all analyses, the ANOVA assumptions of normality and homogeneity of variances were tested using the Shapiro-Wilk and Levene tests, respectively. For parametric data, significant differences ($P=0.05$) among the means were determined by one-way ANOVA followed by post-hoc comparisons using Tukey HSD tests. In instances were variances were found to be heterogeneous, determined using Levene's test, data transformation was used to achieve homogeneity. When transformed data did not satisfy assumptions of ANOVA, randomised permutation ANOVAs were used.

Haemocyte counts for the winter 2013 45 in^3 standard pressure experiment and winter 2014 150 in^3 low pressure experiment, THC data were log transformed to meet ANOVA assumptions and were analysed using a two-way ANOVA with exposure and sample time as factors, followed by post hoc Tukey HSD tests for any significant results. For the summer 2015 150 in^3 standard pressure experiment, data were transformed using Box-Cox analysis which indicated a $\lambda=0.45$, after which data met ANOVA assumptions and were compared using two-way ANOVA with exposure and sample time as factors, followed by post hoc Tukey HSD tests for any significant results. For the winter 2014 150 in^3 standard pressure experiment THC from males and berried females sampled at days 0-14 were sampled serially at days 2 and 14, so values were first checked for equality of variances, then means were compared within each group using mixed-design ANOVAs with sample

time as the within-subject factor and treatment as the between-subject factor. For females sampled at day 120, Welch two-sample t-tests were used for comparison.

Condition indices

Hepatopancreas index data from the winter 2013 45 in^3 standard pressure experiment did not meet ANOVA assumptions, so were compared using a two-way randomised permutation ANOVA with exposure and sample time as factors using 5000 iterations, followed by post hoc Tukey HSD tests for any significant results. HPI data from the winter 2014 150 in^3 low pressure experiment met ANOVA assumptions when tested using a Levene's test and a Shapiro-Wilk test, so were compared using two-way ANOVA with exposure and sample time as factors, followed by post hoc Tukey HSD tests for any significant results. HPI data from the winter 2014 150 in^3 standard pressure experiment were compared using Welch two sample t-tests for between control and exposed treatments for males, berried females sampled at days 0-14 and berried females sampled at day 120. HPI data from the winter 2015 150 in^3 standard pressure experiment met ANOVA assumptions following a log transformation, and were compared using two-way ANOVA with exposure and sample time as factors, followed by post hoc Tukey HSD tests for any significant results.

Bundle index data from the winter 2013 45 in^3 standard pressure experiment met ANOVA assumptions following a Box-Cox analysis and transformation of λ=1.6, so were compared using a two-way ANOVA with exposure and sample time as factors, followed by post hoc Tukey HSD tests for any significant results. BI data from the winter 2014 150 in^3 low pressure experiment met ANOVA assumptions when tested using a Levene's test and a Shapiro-Wilk test, so were compared using two-way ANOVA with exposure and sample time as factors, followed by post hoc Tukey HSD tests for any significant results. BI data from the winter 2014 150 in^3 standard pressure experiment was compared between control and exposed treatments from group 3 lobsters using a Welch two sample t-test.

Embryonic development, larval quantity and quality

Length and width data were tested for normality using the Wilks-Shapiro test and for equality of variances using Bartlett's test and residual versus fit plots. Length data for all three experiments failed the assumption of normality so empirical Box-Cox transformations were applied (Box & Cox 1964). Values of λ for the transformations of length on winter 2013, 45 in^3 standard pressure, winter 2014, 150 in^3 low pressure and winter 2014, 150 in^3 standard pressure experiments were 1.5, 1.8 and 1.6, respectively. Width data for all three experiments passed both normality and equality of variance tests so were not transformed. Data were then analysed using a nested ANOVA with clutch (larvae hatched from the same individual) nested within treatment (control or exposed).

The number of hatched larvae, dry mass and energy comparisons were tested for normality and equality of variance using the Wilks-Shapiro test and Bartlett's test, respectively. All data sets were normal with equal variances and were analysed first with ANCOVA with carapace length as a covariate. Carapace length was a significant factor only for the count data, so these results are reported, and ANOVA was used to compare dry mass and energy.

Larval competency as measured using an elevated temperature and decreased salinity activity test was compared using survival analysis with a Kaplan-Maier estimation and logrank test for trend in GraphPad Prism 6 (GraphPad Software, Inc.).

Scallop Methods

Animal care and experimental design

Commercial scallops (*Pecten fumatus*) used in this study were held at the Institute for Marine and Antarctic Studies, Taroona, Tasmania (IMAS) in a 3400 litre (2 m × 2 m × 0.85 m) tank with *ca.* 10 cm sand substrate with ambient temperature (*ca.* 13°C in 2013 and 2014 experiments, *ca.* 17°C in 2015 experiment) seawater supplied by a flow through system. Scallops were transported to IMAS in plastic bins lined and covered with burlap sacks wetted with seawater (Peterson *et al.* 1996). Upon arrival at IMAS, scallops were tagged with colour-coded and numbered tags (Glue On Shellfish Tags, Hallprint Fish Tags, Australia); randomly assigned to exposure level treatment groups, which were identified by tag colour; measured for shell dimensions and weighed. Scallops were held at IMAS for one week prior to transport to the field site.

Scallops were placed into a large bin (1.2 m × 0.75 m × 1 m) of seawater for transport to the field site. A deck hose was used to pump fresh seawater into the bin to maintain O_2 levels. At the field site, scallops were placed by divers into 1.5 m tall cylindrical enclosures constructed of 2 cm mesh with a 1.2 m diameter floating ring at the top and skirted by a ring of heavy gauge chain at the bottom (Fig. 7). Enclosure bottoms were not meshed, allowing for scallops to be in contact with the seabed. A total of 20 enclosures were placed in two parallel lines of 10, with each enclosure comprising an experimental replicate (Fig. 4). Scallops were held in the enclosures for a two day acclimation period prior to the experiment.

To emulate the exposure animals would receive in a real world setting, pass-bys of the air gun were used for exposure with treatments defined by the number of passes. In the 2013 and 2014 experiments (see below for details), scallops were exposed to 0 (control), 1, 2 and 4 passes. In both experiments, the time required to refill the compressed air cylinders following each air gun run necessitated conducting field work over two days, with passes 0 and 1 conducted on the first day and 2-4 conducted on the second day. In the 2014 experiment, exposure was reduced to 0 and 4 passes. For each pass, the air gun vessel was positioned approximately 1 km from the scallop enclosures with the air gun deployed (Fig. 8). First, a control (0-pass) run was conducted, in which the air gun vessel followed the same approach as the subsequent exposure runs with the airgun deployed and charged, but not fired. Following the control run, divers collected the scallops assigned to the control treatment based on tag colour. Upon retrieval, scallops were randomly assigned to sample points (details below) and placed into a large bin of seawater continually supplied with fresh seawater via the deck hose. After all 0-pass control scallops were collected, the air gun vessel returned to the starting point and began firing the air gun while following the approach toward the scallop enclosures. At the conclusion of the run, divers collected the scallops assigned to the 1-pass treatment, which were assigned to sample times (details below) and placed into the bin of seawater and returned to IMAS.

Following each air gun pass, the air cylinders used to power the air gun required recharging via on board compressors. As this process lasted for several hours, the number of air gun runs that could be conducted was limited, so the 0-pass and 1-pass runs were conducted in one day and passes 2-4 were conducted the following day. Scallops in the 2- and 4-pass treatments were exposed, recovered and transported as described for 0- and 1-pass scallops.

Upon return to IMAS, day 0 scallops were sampled according to the procedure detailed below and a recessing test was conducted on Day 14 and 120 scallops. Following the recessing test, Day 14 scallops were held at IMAS until sampling on day 14, whereas Day 120 scallops were placed into lantern nets, with 4 individuals haphazardly placed into each of the 10 levels of the 2 nets. The nets were placed into bins lined and covered with burlap sacks wetted with seawater and transported to Spring Bay Seafoods in Triabunna, Tasmania (42° 35' 59.65" S, 148° 01' 01.83" E; Figure 9), where the lantern nets were deployed on mussel aquaculture lines that were held submerged at a depth of 10 m. The nets were left undisturbed until they were recovered and returned to the holding tanks at IMAS one week prior to sampling at day 120, to allow for acclimation following transport.

Figure 7. Photograph of scallop enclosure *in situ* at the field site. The float ring at the top and chain ring skirting the bottom kept the enclosure aloft in the water column and the bottom was unmeshed to allow scallops contact with the substrate; parallel lines of rope were used to keep it in place in the event of rough conditions. A total of 20 enclosures were used with each enclosure comprising an experimental replicate.

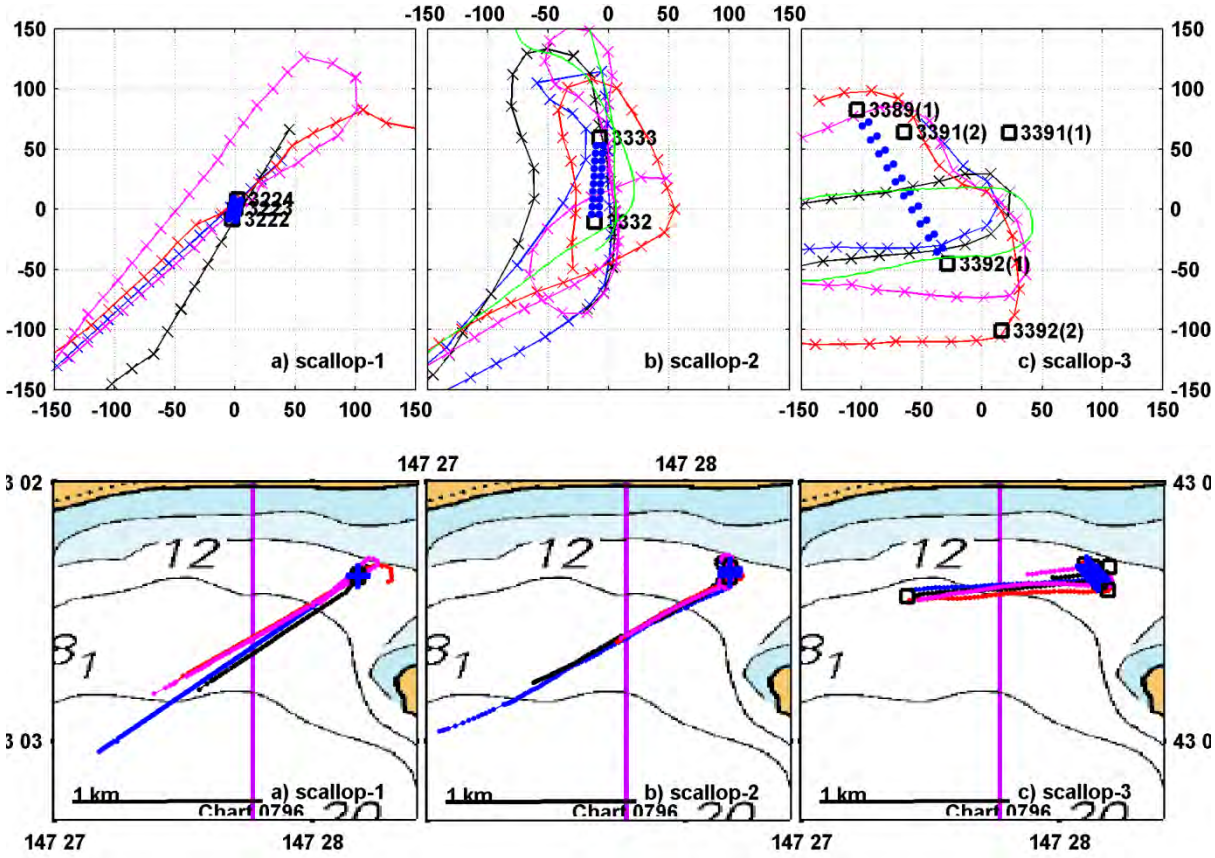


Figure 8. Layout of the three scallop experiments at a small scale (top) and large scale (bottom), with the cages shown by the dots (during experiment 1 the cages were co-located with sea noise recorders), the sea noise logger locations by the black squares, air gun runs: run 1 (blue); 2 (black); 3 (red); and 4 (magenta) shown, with individual air gun shot locations given by the cross along the respective line, and the 2014 and 2015 control runs in green. The numbers on the top panel are the sea noise logger set number. The upper panels show the cage layout with the grid scale in m and the axis arbitrarily scaled to the approximate centre of the line of pots. The lower panels show the larger scale run detail with a) (2013), b) (2014) and c) (2015) referring to the experiment number. The bathymetry contours in the lower panels are from the coast: 5 m; 10 m; 15 m; and 20 m.

2013 experiment

For the first experiment, which was conducted in July 2013 (austral winter), 240 adult scallops were collected by a commercial scallop dredge fishing vessel from the fishery near Ille des Phoques, Tasmania (42° 21' 20.62" S 148° 10' 3.25" E; Figure 9). A 45 in^3 air gun operated at 2000 psi was used for exposure. Scallops were randomly assigned to 4 treatments of exposure levels defined by the number of passes of the seismic air gun: 0-pass (control), 1-pass, 2-pass and 4-pass. To determine whether time was a factor in any observed response, scallops from each of these treatments were sampled at three different points following exposure: 0 days, 14 days and 120 days. Thus, 12 scallops (i.e. 3 sample days × 4 treatment levels) were placed into each of the 20 enclosures at the field site.

On each sample day, the scallops to be sampled, as identified by colour coded tags, were collected from the holding tank and held in a plastic crate that remained immersed in the holding tank. Scallops were haphazardly selected from this crate for sampling until all were done. The sampling procedure (details below) was, in order: total mass, fixation of the total haemocyte count sample, haemolymph pH and refractometer measurements, adductor and shell mass. Scallops not sampled on day 0 were used in recessing tests.

Due to losses from predation and mortality, final sample sizes varied for each treatment and sampling point and are given in Appendix I.

2014 experiment

For the second experiment, conducted in July 2014 (austral winter) using a 150 in^3 air gun operated at 2000 psi, 240 adult scallops were hand collected by divers from Coles Bay, Tasmania (42° 07' 45.07" S 148° 16'03.83" E; Figure 9). Treatment groups and sample times were identical to those of Experiment 1. Scallop sampling occurred according to that of the 2013 experiment except for three variations. First, divers placed video cameras into 10 randomly selected scallop enclosures prior to the seismic vessel passes for behaviour analysis. Cameras were collected along with scallops at the conclusion of each pass. Second, during sampling, two aliquots of haemolymph were frozen in liquid nitrogen for biochemical analysis (details below) as haemolymph was taken for pH and refractive index measurements. Third, the recessing test was repeated on remaining scallops prior to the Day 120 sampling point during the 1 week acclimation period. Final sample sizes for each component of the experiment are given in Appendix I.

2015 experiment

In March 2015 (austral summer), a 150 in^3 air gun was used for the third experiment, in which the number of treatment groups was reduced to two (0-pass and 4-pass) and the number of sample points was reduced to two (14 days, 120 days). For this experiment, 80 adult scallops were hand collected by divers from Coles Bay, Tasmania at the same site as the 2014 experiment, and 4 scallops (i.e. 2 sample days × 2 treatment levels) were placed into each enclosure. Similar to the 2014 experiment, divers placed video cameras in 10 of the enclosures just prior to seismic vessel passes and recovered the cameras once the pass was concluded. The recessing test was conducted after initial exposure and the righting test was conducted just prior to sampling at Day 14. Both tests were intended to be conducted prior to the Day 120 sample; however, all scallops (control and

exposed) were dead upon retrieval. All other sampling was performed as in the 2013 experiment. Final sample sizes for each component of the experiment are given in Appendix I.

Figure 9. Scallop experiment locations. 1) IMAS; 2) Blackjack Rocks, field site; 3) Spring Bay Seafoods, Triabunna, mussel lease where day 120 scallops were held; 4) Ille de Phoques, collection site for 2013, 45 in^3 experiment; 5) Coles Bay, collection site for 2014 and 2015, 150 in^3 experiments.

Sampling

At each sample day in all three experiments and throughout the post-exposure holding period, scallops were assessed for mortality, which was detected by one of two ways: through observation of abnormal positioning (i.e. inverted on the substrate, not recessed for an extended period, leant up against the side of the tank, etc.) during periodic (i.e. at least 3 times per week) monitoring of the scallop tank or discovery during sampling. Any observed mortalities were rounded to the next sampling point, i.e. a dead scallop discovered at day 10 was considered dead at the day 14 sample

point. For the day 120 scallops, once scallops were transported to the mussel lease, mortality was not assessed until the scallops were returned to IMAS prior to sampling. Mortality rate was determined by comparing the total number of dead scallops from each treatment. Only mortalities observed following recovery were considered, i.e. losses due to predation or unrecovered animals were not counted in the analysis.

At each scheduled sample point in all three experiments, all individuals within the four treatment levels (two treatment levels in the 2015 experiment) were destructively sampled. First, scallops had excess water drained from the mantle cavity and the total weight and shell dimensions were recorded. Next, from each scallop, 2.5 ml of haemolymph was drawn from the pericardial sinus using a pre-chilled syringe fitted with a 26 gauge needle. This sample was divided into 3 aliquots: a 500 µl aliquot for immediate analysis, a 500 µl aliquot that was added to a centrifuge tube pre-filled with 500 µl anticoagulant (Baker's formol calcium: 2% NaCl, 1% calcium acetate, 4% formaldehyde) and a 1500 µl aliquot that was centrifuged at 3,000 g for 3 minutes after which 1000 µl of supernatant was transferred into a cryovial and snap frozen in liquid nitrogen for later biochemical analysis.

In all three experiments, the 500 µl aliquot of fresh haemolymph was immediately measured for pH (Testo 205 pH meter) and refractive index (HI 96801, Hanna Instruments) with a conversion factor of SG=0.0041 x Brix + 1.0 and the 1:1 (v/v) mixture of haemolymph and anticoagulant was used for total haemocyte counts using an improved Neubauer haemocytometer under 40x magnification. In the 2014 experiment, the 1000 µl centrifuged supernatant sample was shipped, frozen on dry ice, to Diagnostic Services at the Atlantic Veterinary College, University of Prince Edward Island, Canada, and analysed using a Cobas c501 automated biochemistry analyser (Roche Diagnostics Corporation) for a full blood profile consisting of the electrolytes (mmol l^{-1} = mM) sodium (Na), chloride (Cl), potassium (K), magnesium (Mg) and bicarbonate (bicarb); minerals (mmol l^{-1}) calcium (Ca) and phosphorus (P); metabolites (mmol l^{-1} = mM) glucose (Gluc), lactate (Lact), cholesterol (Chol), triglyceride (Trig), total protein (TP, in g l^{-1}) and uric acid (Uric, in µmol l^{-1}); enzymes (U l^{-1}) lipase (LIP), amylase (AMY), alanine (ALT) and asparate (AST) aminotransferases, alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT).

Finally, the shell valves were separated, the soft tissue was removed and the adductor was weighed. These weights and measurements were used to derive five condition indices: mass-to-length (tissue mass · shell length^{-1}; Hannem *et al.* 2010a), mass-to-volume ([total mass · (shell length · shell width · shell height)$^{-1}$] ·10,000; Maguire *et al.* 1999), relative tissue mass ([tissue mass · total mass^{-1}] · 10); percent adductor mass ([adductor mass · total mass^{-1}] · 100; Kleinman *et al.* 1996), relative adductor mass ([adductor mass · tissue mass] · 10; Kleinman *et al.* 1996). The mass-to-length, percent adductor mass and relative adductor mass indices were modified from the cited sources in that wet tissue masses were used in place of dry tissue masses.

A recessing reflex test following the method of Maguire *et al.* (1999; 2002) was conducted on the day 14 and 120 scallops. Starting from when the scallops were placed into the holding tank following exposure, scallops were visually assessed for recessing 3-4 times daily at approximately 6 hour intervals, with recessing defined as having an upper valve even with the substrate level. To ensure consistent assessment of recessing, the same researcher performed all assessments. Once a scallop was observed to have recessed into the substrate, it was collected and the time taken to

recess (in hours) was recorded. In all 3 experiments, the recessing test continued until all scallops had recessed, which was under 5 days in all cases. In the 2014 experiment, this test was also conducted a second time, prior to the day 120 sample point.

A righting reflex test was conducted for the summer 2015 experiment as described by Minchin *et al.* (2000a; 2000b), in which scallops were placed on the sand substrate of the holding tank with the top valve down. When a scallop righted itself, its tag and elapsed time were recorded. All scallops righted themselves over the course of approximately 30 mins.

Video recordings were made during seismic exposure in the 2014 and 2015 experiments to allow for the analysis of scallop behaviour. Recordings were divided into 3 categories: pre-, intra- and post-exposure segments, with the first 5 mins of pre-exposure time and the last 5 mins of post-exposure time disregarded due to the influence of divers deploying and retrieving video cameras. For each segment, all visible scallops were observed and two sets of behavioural data were recorded. For the first set of behavioural data, the observed behaviours were classified into two categories, classic and non-classic, with the former defined according to Wilkens (2006), encompassing visual behaviours, e.g. reflexive closure response to shadow or movement; “coughs” used to irrigate the mantle cavity; valve closures characterized by mantle velum retraction and valve adduction; and locomotory behaviours, such as swimming or repositioning. Any behaviours not encompassed within the classic behaviour category were classified as non-classic. The second analysed behaviour was tentacle extension, with tentacles recorded as either “extended,” “partially extended,” or “retracted” for the duration of the pre-, intra- and post-exposure time categories for the 2014 and 2015 experiments. It is important to note that the pre-exposure periods differ somewhat between the 1-pass treatment and the higher exposure treatments, in that the 1-pass scallops were naïve to any air gun exposure during the pre-exposure period, whereas 2- and 4-pass scallops had been exposed during the intra-exposure period of the previous treatments.

Statistics

To evaluate cumulative mortality in the 2013 and 2014 experiments, the total number of mortalities from each treatment was compared using a binomial regression. The analysis was restricted to the 2013 and 2014 experiments, as all scallops died while deployed on the mussel lease prior to the day 120 sampling point for both 0- and 4-pass treatments in the 2015 experiment.

Haemocyte count data for the 2013 experiment initially failed tests for both equality of variance, using Levene’s test, and normality, using Shapiro-Wilk test, which was corrected with a log transformation, after which data met assumptions and were compared using a 2-way ANOVA with air gun passes and sample time as factors and $\alpha=0.05$, followed by a Tukey HSD post hoc test for significant results. The results of the 2014 experiment initially failed the test for normality, which was corrected using a square root transformation, after which data was analysed as in the 2013 experiment. Data for the 2015 experiment were compared using a Welch Two Sample t-test.

Haemolymph pH data for the 2013 experiment did not meet normality assumptions, so a two-way randomised permutation test ANOVA with 5000 iterations was used following Manly (2007), with passes and sample time as factors and $\alpha=0.05$, followed by post hoc Tukey HSD tests for any significant results. For the 2014 experiment, data met assumptions of normality and homogeneity of variance, so a two-way ANOVA with passes (0-pass, 1-pass, 2-pass, 4-pass) and sample time (0, 14,

120 days) used as factors and α=0.05. Significant results were analysed using a Tukey HSD post hoc test. Refractive index data from the 2013 experiment met ANOVA assumptions, so a two-way ANOVA was performed. Refractive index data for the 2014 experiment did not meet ANOVA assumptions, so a randomised permutation test ANOVA with 5000 iterations was used, followed by post hoc Tukey HSD tests for any significant results. For the 2015 experiment, Welch t-tests were used to compare both pH and refractive index data sets, as comparison was between two treatments (0-pass and 4-pass) at only one sample point (14 days).

Biochemistry data from the 2014 experiment was tested for normality and for homogeneity of variances. For parametric data, significant differences (P=0.05) among the means were determined by two-way ANOVA with passes and sample time as factors and α=0.05, followed by post-hoc comparisons using Tukey HSD tests. For data failing to meet ANOVA assumptions, a randomised permutation test two-way ANOVA was performed (Manly 2007) with passes and sample time as factors and α=0.05, and post-hoc pairwise comparisons with Bonferroni correction used to evaluate significant results.

Comparisons of behavioural analysis were conducted on the rate (incidences per unit time) of observations of classic and non-classic behaviours. Rates were used as the observational period differed between the scallops, thereby complicating the use of conventional count based models. Non-classic behaviour was only observed in exposed scallops hence the non-classic and classic behavioural modes were analysed separately using a generalised linear model with the number of exposure passes, the year and temporal category (pre-, intra- and post-exposure) as explanatory variables. All explanatory variables were categorical, this decision was made for the number of exposure passes to allow for non-linearity in the exposure relationship.

Tentacle extension was compared for each treatment by summing the duration each individual scallop spent in each of the three states of tentacle extension. This sum was then converted into a proportion of total time of each temporal category, and multinomial regression was used to analyse the behavioural modes (two options, since the proportions add to one) as a function of the year, treatment and phase.

Recessing reflex data from all 3 experiments was analysed using Kaplan-Meier survival analysis of the time-to-recess for each individual and compared using log-rank (Mantel-Cox) test with α=0.05, followed by multiple comparisons with Bonferroni correction and a family wise error rate of 0.05. The righting reflex test from the 2015 experiment was analysed using Kaplan-Meier survival analysis and compared using log-rank (Mantel-Cox) test with α=0.05. Both of these data sets were analysed using GraphPad Prism 6.

All condition indices for the 2013 and 2014 experiment were tested for normality using Shapiro-Wilks test and for homogeneity of variance using Levene's test. Data for all five indices in both years passed Levene's test. Relative tissue mass, percent adductor mass and relative adductor mass failed the normality test for 2013 and were transformed using the Box-Cox transformation (Box and Cox 1964) with lambda values of 0.65, 0.85 and 0.19, respectively. For the 2014 experiment, mass-to-volume, relative tissue mass and percent adductor mass failed the normality test and were Box-Cox transformed using lambda values of -1.22, 0.7 and 1.3, respectively. Following transformation, data were compared using 2-way ANOVA with air gun passes and sample time as factors with α=0.05, followed by Tukey HSD post hoc tests for any significant results. All indices for the 2015

experiment met the assumption for normality when tested with the Shapiro-Wilk test and were compared using Welch t-tests with α=0.05.

Except where noted otherwise, all statistical comparisons were conducted using R v3.1.3 (The R Foundation for Statistical Computing).

Ethics and permits

All research was conducted in accordance with University of Tasmania Animal Ethics Committee permit #A13328. Field work was conducted in accordance with Tasmania Department of Primary Industries, Parks, Water and Environment permits #13011 and 14038.

General Results

Temperature

The temperatures recorded during each experiment are shown on Figure 10 where the time base has been zeroed to 00:00 hours (EST) on the first day of each experiment. Temperatures recorded at the experimental sites were comparatively stable during experiments. The temperatures recorded by the receiver located near the beach during the 2014 experiments oscillated considerably. The mean temperatures for each experiment are listed in Table 4.

Table 4. Mean seabed water temperatures during lobster and scallop experiments.

Experiment	Mean ºC
2013 lobster	11.6
2013 scallops	11.8
2014 beach	10.5
2014 scallops	10.5
2014 lobster	11.6
2015 lobster	17.7
2015 scallop	17.4

Air gun geophone signals

The geophone signals measured the vertical and horizontal ground movement that occurred in response to the air gun signal. From the velocity measurements ground acceleration was derived and has been used as the primary unit for ground motion here, since acceleration is more pertinent to noise impact effects. The acceleration applied to an animal will determine shear forces within tissues and between internal tissue and density discontinuity boundaries (ie. some sensory organs). High shear forces within an animal may cause mechanical trauma.

The ground motion was considered more pertinent to scallops as they were directly coupled into the seabed and so would have received all ground accelerations whereas the lobster legs would act like springs, partly decoupling the lobster body from the ground shaking.

An intense pulse in the water column will excite the seabed so that energy travels within the seabed and along the interface between the seabed and the water via three mechanisms:

1. As a result of the waterborne signals' particle acceleration directly driving the local seabed;
2. As a high-speed ‘head wave’ that travels at the seabed compressional wave speed, which is faster than the speed of sound in water. Head waves arrive before the water borne pressure signal and typically travel at different speeds through different sea bed layers so multiple head wave arrivals may be found close to the seabed;

3. As a low-speed interface wave known as Scholte or Rayleigh waves. These waves only exist when the seabed (or some layer within it) is solid enough to support the propagation of shear waves. In most seabed types Scholte or Rayleigh waves travel slightly slower than the seabed shear speed, which is usually slower than the speed of sound in water so they arrive after the water borne energy.

Examples of the waterborne signal waveform, the vertical and horizontal ground acceleration and the magnitude of the ground borne motion (vector sum of vertical and horizontal motion components) are shown in Figure 11. The shaking occurred over ~ 0.070 s (mean of 490 signals, measured by receiver 3389 in the 2015 experiments, of the time taken for 90% of the ground energy to pass) and reached high acceleration values. The trend of this ground vibration is shown in Figure 12 with logarithmic range and the maximum peak ground-motion magnitude, given as decibel values ($10 \times \log_{10}$(linear value/1) with a reference value of 1 ms^{-2}). There was a steady increase in the decibel value of the ground motion as the source approached. There were some high level outliers, with the maximum measured ground borne acceleration magnitude of 68 ms^{-2} for a signal at 37 m range (~18 dB in Figure 12), which is very high (approximately 7 G).

In the experiments here it appears the ground responded to the waterborne signal directly stimulating the seabed, owing to high frequency energy in the ground acceleration above what would be expected from Scholte, Ralyeigh or headwaves.

The ground vibration was evident on the cameras used in scallop experiments, the cameras would shake vigorously on passage of the air gun signal (as discerned by the camera audio) then a small plume of sediment would arise from the seabed.

The ground motion is referred to as ground roll here and is presented in units of maximum absolute magnitude of acceleration per air gun shot, in linear (ms^{-2}) or dB values (dB re $1ms^{-2}$). Different ground roll units were explored but the simplest has been chosen here - the biggest acceleration experienced during passage of an air gun signal. This unit does not directly account for exposure history from multiple air gun signals or the time history during a single air gun signal. As yet there are no techniques for measuring ground roll exposure history for benthic marine fauna. We did trial a similar approach as used for SEL and SEL_{cum} calculations to quantify each air gun signals' ground roll magnitude, but as this requires careful consideration as to what is a relevant biological measure for ground roll and that maximum ground roll magnitude was tightly correlated with its SEL equivalent, alternative units have not been used here. When using the same equations as given in McCauley *et al*. (2003) to give an SEL equivalent for ground roll (including accounting for noise) the maximum ground roll magnitude (dB re 1 ms^{-2}) and its SEL equivalent (dB re 1 m^2s^{-3}) were tightly correlated (r^2 = 0.98) implying the maximum ground roll magnitude described the time exposure measure.

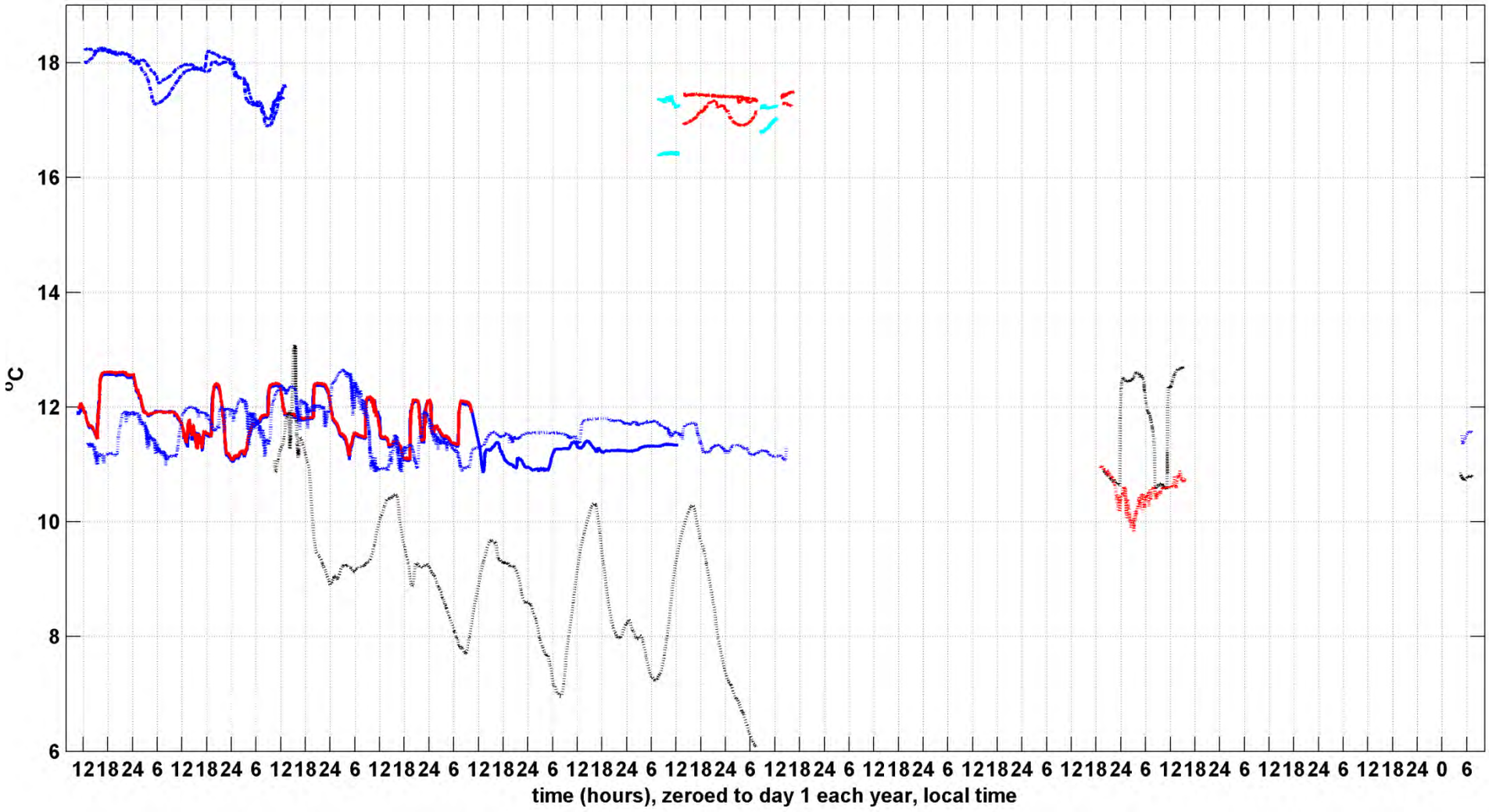


Figure 10. Seabed temperature data from all experiments with the time base zeroed to 00:00 hours (EST) on the first day of each experimental period. The curves are coded as: 2013 lobster - blue, solid line; 2013 scallops - red solid line; 2014 beach - black dotted line; 2014 lobster - blue dotted line; 2014 scallops - red dotted line; 2015 lobster - blue dot-dashed line; 2015 scallop - red dot-dashed line; 2015 south of Betsy Island - cyan solid line.

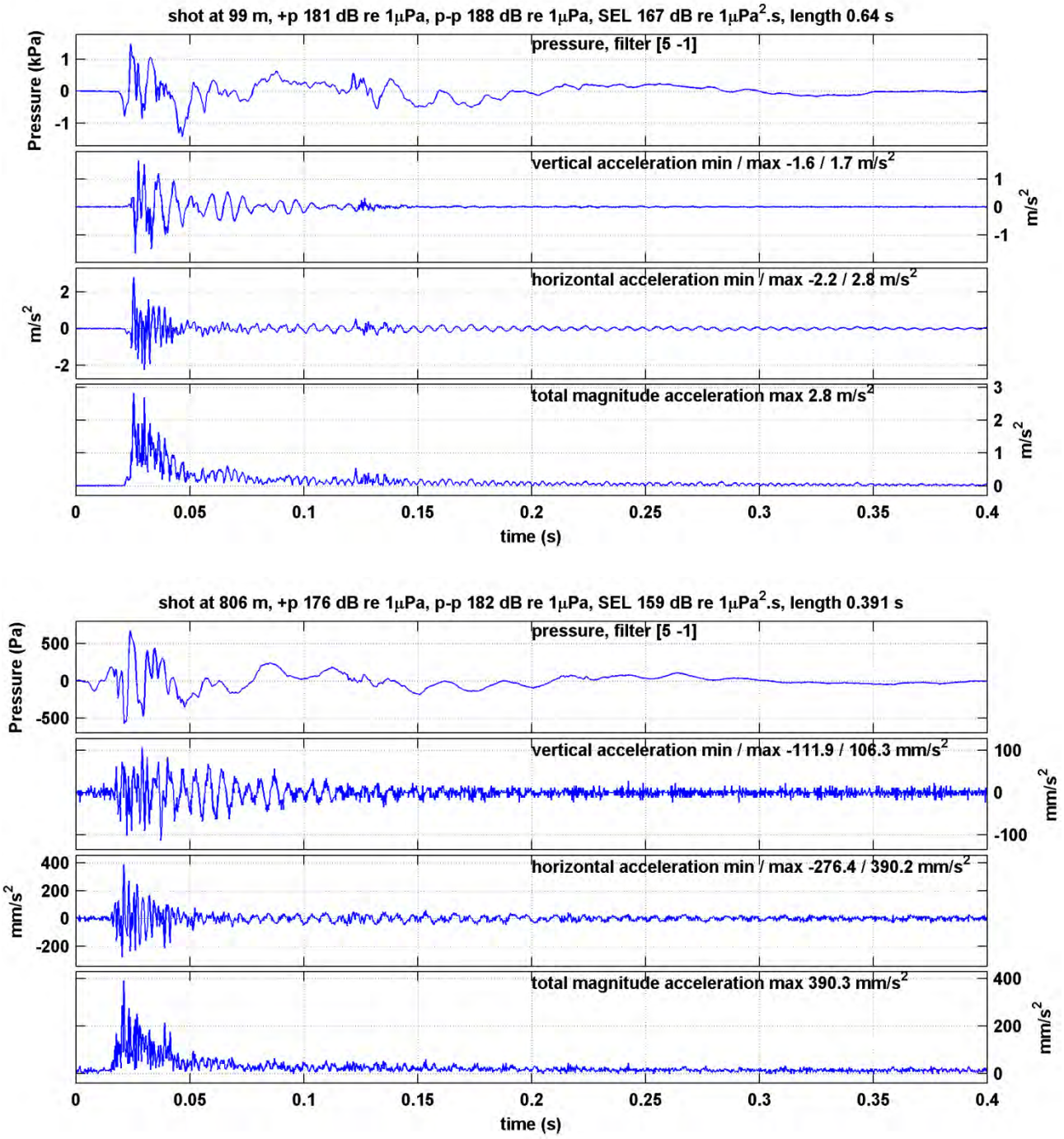


Figure 11. Example of air gun signal waveform, vertical and horizontal ground acceleration and the magnitude of ground acceleration, for signals at 99 and 806 m range during the first air gun pass in scallop experiment 3.

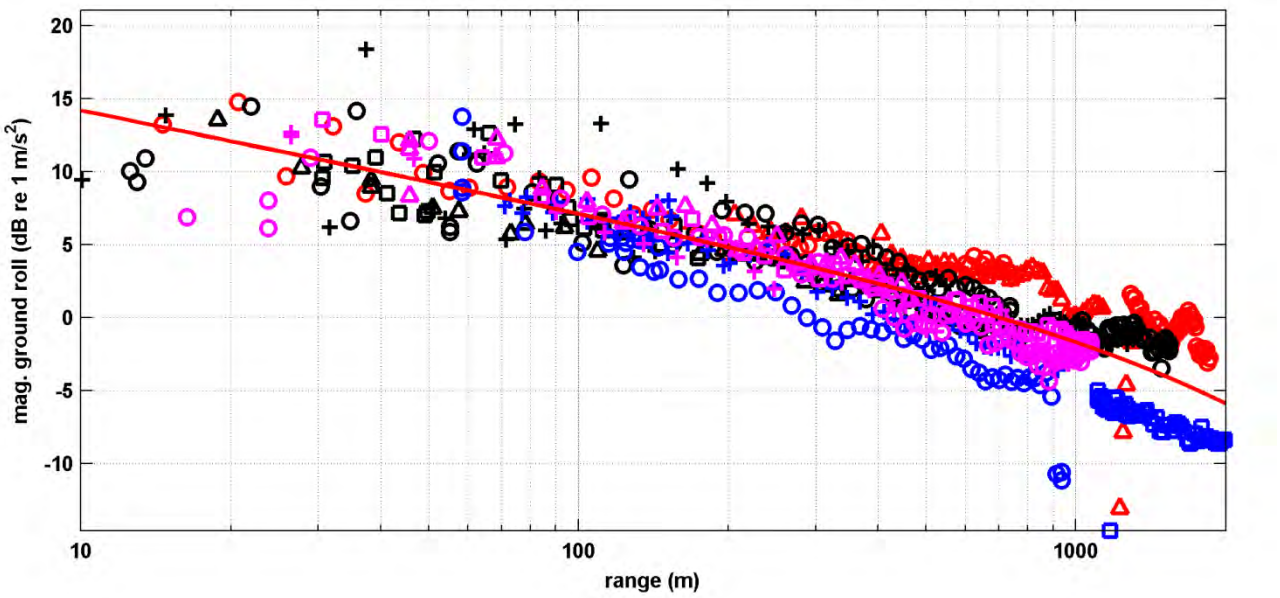


Figure 12. Maximum (peak) ground borne acceleration values, given in decibels, from all 2014 and 2015 measures with a curve of best fit shown.

Air gun pressure signals

Examples of measured air gun levels (sound exposure level and peak-peak) from the 2015 experiments, which was representative of transmission during experiments in other years, are shown in Figure 13. There were considerable differences in transmission of the air gun signals at ranges greater than 100 m when comparing the lobster and scallop locations. The lobster experimental area was a limestone platform as opposed to the scallop site being over sand. Limestone seabeds are known to cause high sound transmission losses, which is evident in the plots for most of the received signals at greater than 100 m range in the lobster experiments. At ranges less than100 m the received signal levels were similar between scallop and lobster measures. During one lobster measurement shown in Figure 13 the receiver was placed on a sandy seabed at 1 km from the experimental site, such that the air gun was at this point operating over a sand seabed. This receiver had measured air gun levels similar to the trend seen from the scallop measures which were all made over sand.

As the lobster and scallop experiments had 2 to 4 noise loggers and 10 to 20 pots or cages, the received sound exposure level and peak to peak level for all shots at all pot/cages in all experiments have been estimated and experimental exposures given as statistics based on the spread of values across the pots/cages.

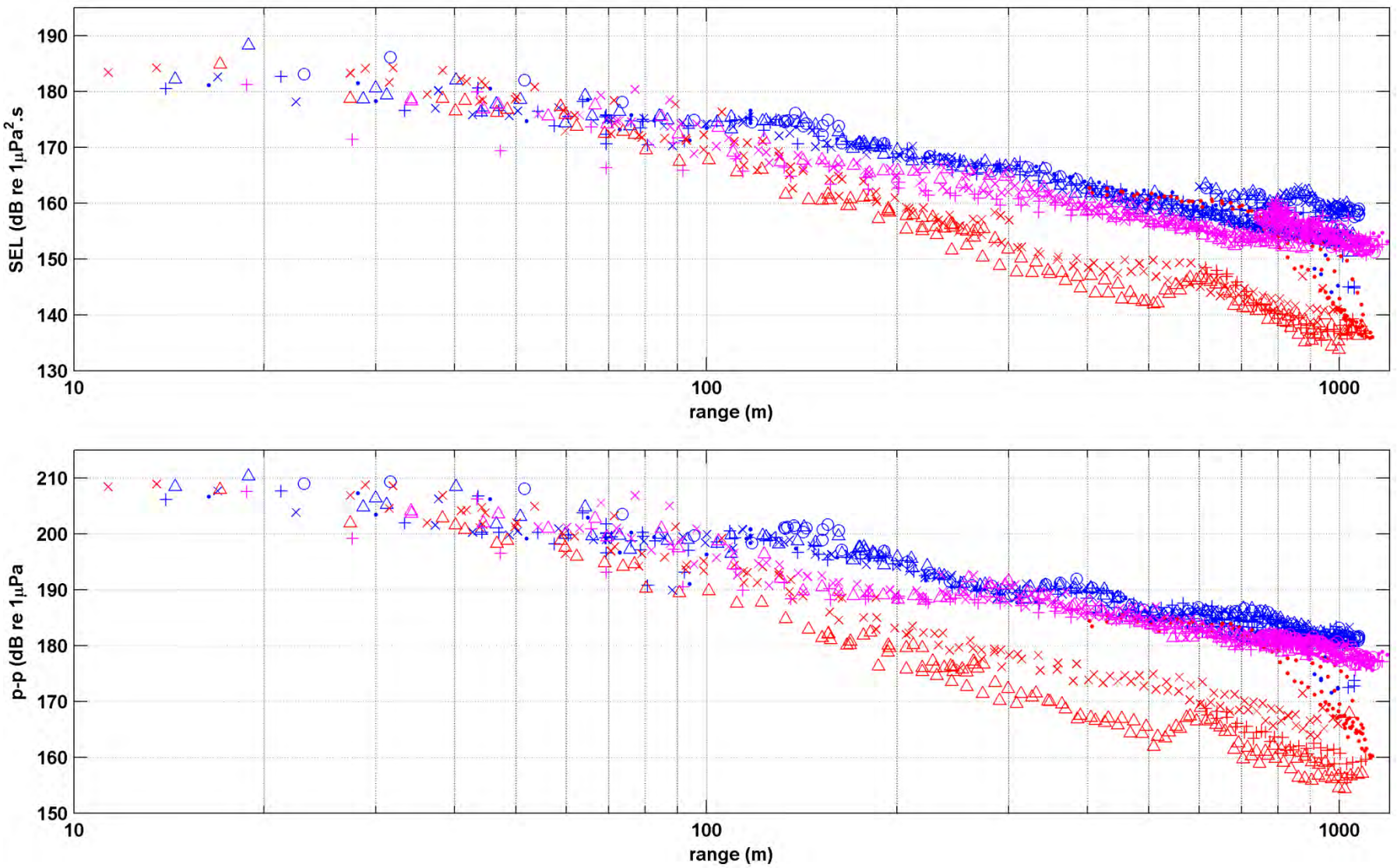


Figure 13. Measured SEL (top) and peak-peak (bottom) values for 2015 experiments. The blue points are scallop runs, the red points lobster runs and the magenta points are measures made in 36 m water just south of Betsy Island.

Lobster exposures

Each lobster experiment involved multiple lobster pots (20, 20 and 10 for the winter 2013 45 in^3, winter 2014 150 in^3 low pressure and winter 2014 150 in^3 standard pressure experiments respectively) spaced along a line extending100-250 m north-south. Thus measurements from the sea noise loggers were used to build relationships of received level (peak to peak and sound exposure level or SEL) for the air gun with range and to use this relationship to estimate all fired air gun signal levels at each lobster pot. The estimated received levels were used in a statistical fashion for all pots to define the exposure regime for the respective experiment, based on levels received at each pot.

A Curtin air-gun source model was used to estimate source levels (at one-m) of the air gun configurations used, which for the winter 2013 45 in^3 standard pressure, winter 2014 150 in^3 low pressure and winter 2014 150 in^3 standard pressure experiments respectively were: peak-peak 223, 224 and 227 dB re 1µPa; and SEL 200, 203, 205 dB re 1µPa2·s . A curve was fitted to the measured levels (peak-peak and sound exposure level independently) of the 150 in^3 standard pressure data, using: a) the mean value in logarithmic range bins; and b) of the form

$$RL = a\log_{10}(R) + b * R + SL$$

where *RL* is received level, *R* is range, *SL* is the (fixed) source level and a & b are values derived from the data. The measured curve a) above described the anomalies in the transmission for the site (due to environmental factors) but was less accurate at ranges < 20 m where the data was scarce. For peak-peak and SEL estimates the two curves agreed over the range 10-20 m so a hybrid curve was used, with ranges < 20 m using the curve b) and ranges > 20 m using curve a). The curve was adjusted for the difference in source level according to the air gun source model for the 45 in^3 and 150 in^3 low-pressure sources to give six sets of curves to predict peak-peak and SEL for the three sources. These curves are shown on Figure 14.

The range of source to receiver (pot) was then used to estimate received level (peak-peak and SEL) at each pot during each experiment from which the statistics given in Table 5 were derived. The cumulative SEL (SEL_{cum} or sum of sound exposure values in linear units of all air gun shots received, expressed in dB values) were calculated for each pot, with the median and maximum SEL_{cum} values derived using data for the different pots. Maximum exposures received depended on pot proximity to the air gun, which was random amongst experiments, but the cumulative sound loading, or total dose of sound received, was highest for the 150 in^3 standard pressure psi experiment as given by the number of signals which exceeded set thresholds and the median SEL_{cum} amongst pots (Table 5). The distribution of peak-to-peak and SEL levels for the lobster experiments are given in Figure 15.

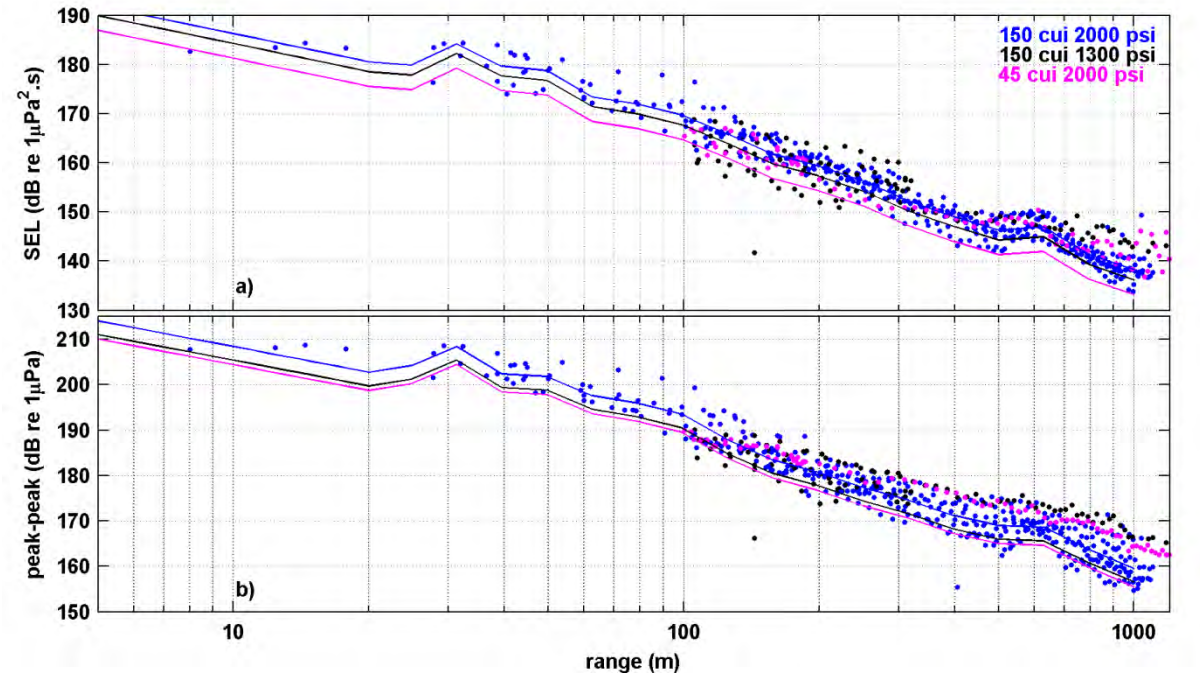


Figure 14. For lobster experiments, quantification of sound exposure by range between the air gun and lobster pots in the winter 2013 45 in^3 standard pressure, winter 2014 150 in^3 low pressure and winter 2014 150 in^3 standard pressure air gun exposure experiments. Sound level is expressed in received sound exposure level (a) and received peak-peak level (b) in the three experiments with range expressed logarithmically.

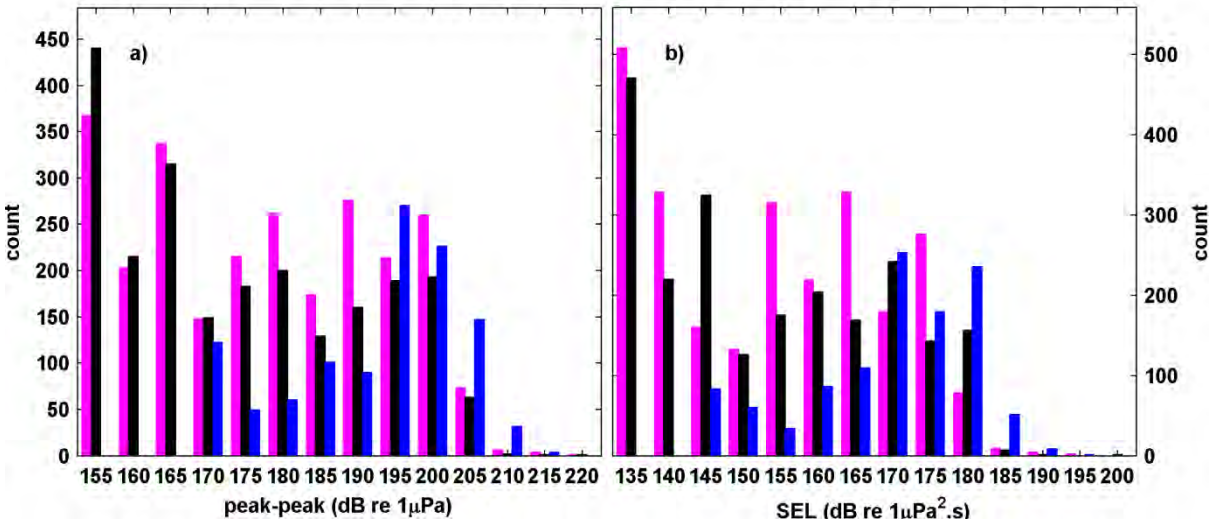


Figure 15. Distribution of estimated levels at all lobster pots for the three experiments (winter 2013 45 in^3 standard pressure magenta, winter 2014 150 in^3 low pressure black and winter 2014 150 in^3 standard pressure blue), with a) peak to peak and b) SEL.

Table 5. Calculated exposure values for the lobster experiments. Given are: maximum peak-peak (pp, dB re 1µPa); number of signals within 3 dB of maximum pp; number of signals > 200 dB re 1µPa pp; maximum sound exposure level (SEL, dB re 1µPa^2·s); SEL within 3 dB of maximum SEL; number of signals > 180 dB re 1µPa^2·s SEL; maximum cumulative SEL (SEL$_{cum}$, dB re 1µPa^2·s); median SEL$_{cum}$.

	Max PP	**Shots PP within 3 dB max**	**Shots with PP > 200**	**Max SEL**	**Shots SEL within 3 dB max**	**Shots with SEL > 180**	**Max SEL$_{cum}$**	**Median SEL$_{cum}$**
45 in^3 2000 psi	209	2	13	186	2	3	192	191
150 in^3 1300 psi	210	1	11	189	1	7	193	192
150 in^3 2000 psi	212	3	38	190	3	25	199	197

Scallop exposures

Examples of measured air gun signal sound exposure levels and ground borne acceleration magnitude (expressed in dB format) recorded during the scallop exposures are shown on Figure 16 along with fits to the data. The measured SEL values showed several features of: 1) at short range (< ~ 150 m) the 150 in^3 source was higher in level at a given range than the 45 in^3 source but at long range (~ > 150 m) the 45 in^3 and 150 in^3 sources followed similar trends; 2) there was high variability in level at short range; 3) there was a bathymetry step at around 1.2 km to the SW of the receiver locations (Fig. 8) which caused an increased loss rate compared with shorter ranges; and 4) there was a slight difference in the received level at > 200 m for the SW versus east approach tracks shown on Figure 8. The 150 in^3 air gun produced greater energy at low frequencies compared with the 45 in^3 air gun, but in the shallow water here this energy dissipated faster with range than the higher frequency air gun energy, hence beyond a certain range, around 100-200 m here, the received levels for the two chamber sizes showed similar loss-with-range trends. The high variability at short range (< 50 m) would be due to position error from the GPS precision combined with predicting the air gun location behind the source vessel GPS aerial. The differences in measured signals at longer range, while significant, mattered less to the exposure received by scallops at the cages, as this was dominated by air gun shots at shorter range (say < 200 m).

As scallops were sampled according to a regime of the number of passes of an air gun run (0, 1, 2 and 4 passes), with each pass having between 51-167 shots (median 65, see

Table 2) and only three to four noise loggers were set to monitor exposure over 20 scallop cages, then similar to the lobster experiments described above we have estimated exposures at each cage and presented statistics of the mean exposure per sample regime. To estimate exposures per cage for the pressure signal or waterborne energy, curves of the form:

$RL = a * \log10(Range) + b * Range + c$

with *RL* = received level in appropriate units, *a* = constant, *Range* in m, and *b* & *c* constants, were fitted to the appropriate data sets of 45 and 150 in^3 sources for peak-peak and SEL. The value of *c*, which is the source level (level emitted by the source at one m range if it were a point) was allowed to float (i.e. derived from the data) for long range signals or fixed at the known source level (45 and 150 in^3 sources 2000 psi, as defined in the lobster section above) for short range signals. For a measure (peak-peak or SEL) the short and long range curves matched well, with cross over points defined as 340 m for peak-peak curves and 100 m for SEL curves. The values derived (or used) in each curve type and the correlation coefficients of the curves (r^2=0.9 to 1.0 for pressure values, r^2=0.78 for geophone data) are listed in Table 6. To estimate received air gun shot levels at each cage the geometry of cage to air gun shot location was used to calculate the slant range of each shot for each scallop cage. The fixed source level curve was used to estimate the respective signal parameter out to the range it crossed the floating source level curve, then the floating source level curve was used beyond this range, for the respective source and unit of measure.

Table 6. Constants for fits generated of measured scallop air gun signal levels (peak-peak and sound exposure level for 45 and 150 in^3 source, and geophone data for absolute magnitude of ground roll). The values *a*, *b* and *c* are either derived from the data (all values of a & b) or pre-set (values c in fixed fits). The correlation coefficient for all fits is given (r^2).

Source	*a*	*b*	*c*	r^2
45 in^3, peak-peak, floating	-8.098	-0.0033	206.7	0.90
45 in^3, SEL, floating	-6.699	-0.0130	186.3	0.93
45 in^3, peak-peak, fixed	-15.247	0.0022	223.0	0.97
45 in^3, SEL, fixed	-12.718	-0.0084	200.0	0.95
150 in^3, peak-peak, floating	-11.489	-0.0071	222.2	0.92
150 in^3, SEL, floating	-10.838	-0.0124	196.7	0.96
150 in^3, peak-peak, fixed	-13.929	-0.0042	227.0	0.97
150 in^3, SEL, fixed	-15.067	-0.0074	205.0	1.03
Geophone, all data (dB re 1 ms^{-2})	-6.877	-0.0021	21.1	0.78

Ground roll measured by the geophones showed comparatively high levels of acceleration received during air gun passage, up to the highest outlier of 68 ms^{-2} (34 m range) for the absolute magnitude of the three sensors, from a mean background noise value of 0.012 ms^{-2} for this measure with no air gun activity. The ground roll measures showed high variability at a given range, most likely due to difference in the seabed type and sand layer thickness between receiver locations. Given the high variability in measures the predictive curves for the 45 and 150 in^3 sources were not differentiated and all data was used to generate a loss relationship with range, as shown on Figure 16b. The parameter values of the fitted curves are given in Table 6.

The calculated exposures for the different scallop experimental regimes used the fitted curve given in Table 6 and Table 7. The ground roll values are presented as minimum and maximum values of the maximum magnitude of ground roll predicted using all seismic signals for each exposure regime, across all scallop cages for that experiment, using the fitted curve of Table 7.

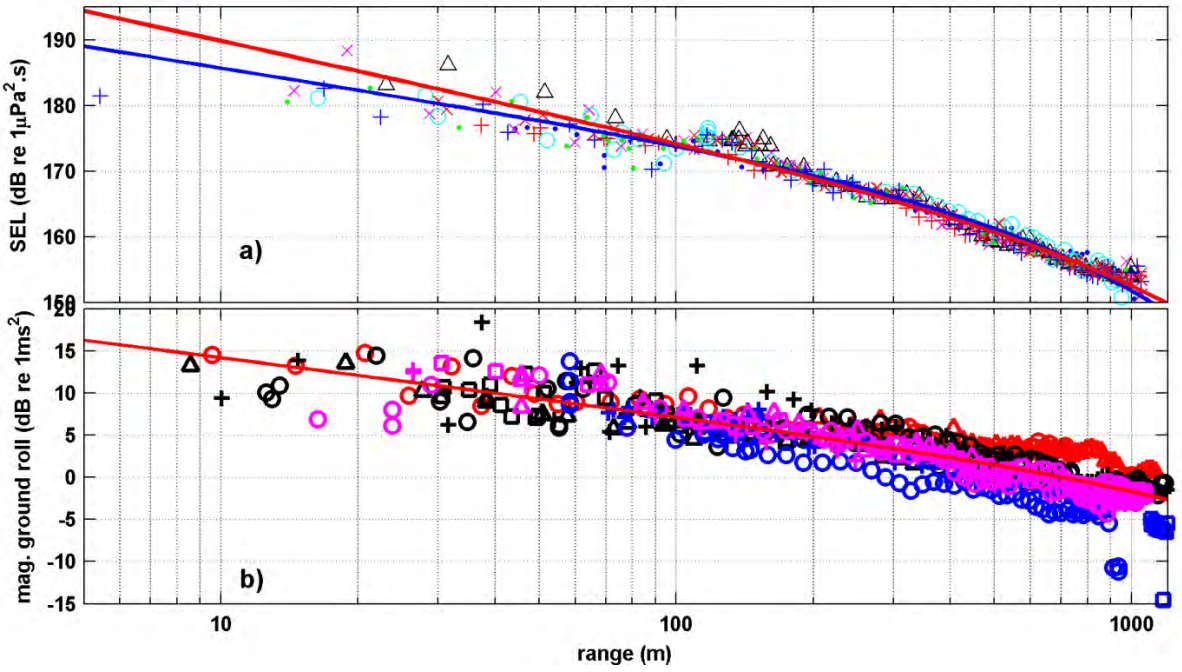


Figure 16. Sound exposure level and ground roll magnitude from summer 2015 150 in^3 scallop exposure experiment showing: a) Measured sound exposure with the fitted curves shown (red is fixed source level, blue is floating source level), and b) Magnitude of total ground roll expressed in dB format with the single fitted curve using data from all three scallop experiments. The different colours represent different receiver locations, the symbols the air gun run.

Table 7. Calculated exposure values for the scallop experiments. Given are: maximum peak-peak (pp, dB re 1µPa); number of signals within 3 dB of maximum pp at any cage; number of signals at any cage > 190 dB re 1µPa pp; maximum sound exposure level (SEL, dB re 1 µPa^2·s); maximum shots / cage within 3 dB of maximum SEL; maximum shots / cage with signals > 180 dB re 1µPa^2·s SEL; maximum cumulative SEL (SEL$_{cum}$, dB re 1 µPa^2·s); median SEL$_{cum}$ across cages; number of shots / treatment; estimated minimum magnitude ground roll / treatment (ms^{-2}); estimated maximum magnitude ground roll / treatment (ms^{-2}).

Experiment	Max PP	Shots within 3 dB max PP	Shots > 190 PP	Max SEL	Shots within 3 dB max SEL	Shots > 180 SEL	Max SEL$_{cum}$	Median SEL$_{cum}$	# shots	Min Geo	Max Geo
E-1 45 in^3 pass 1	191	40	14	181	3	1	189	189	167	0.29	37.22
E-1 45 in^3 pass 1 & 2	191	63	23	181	5	1	191	191	226	0.29	37.27
E-1 45 in^3 pass 1-4	191	148	52	181	8	2	194	194	393	0.29	37.57
E-2 150 in^3 pass 1	212	2	40	187	2	5	193	192	128	0.27	31.60
E-2 150 in^3 pass 1 & 2	213	2	71	188	2	8	195	194	195	0.27	35.37
E-2 150 in^3 pass 1-4	213	3	151	188	4	19	198	198	309	0.27	36.39
E-3 150 in^3 pass 1	213	1	26	188	1	3	191	188	54	0.68	35.54
E-3 150 in^3 pass 1 & 2	213	2	61	188	2	6	195	193	115	0.68	36.60
E-3 150 in^3 pass 1-4	213	2	140	188	2	6	197	196	251	0.67	36.60

Comparative commercial seismic exposures

The exposure levels measured in the present study were compared to levels of a hypothetical seismic source modelled on field measurements of full-scale arrays as reported by Anon (2011), McCauley *et al*. (2016) and data sets of seismic survey configurations and their transmission with range held by an author (RDM, unpublished data). The hypothetical seismic source considered was:

- a 3065 in^3 source operating in 3D mode;
- two sources operated alternatively, each source located centrally 50 m either side of the sail-line;
- four streamers spaced 100 m apart and symmetric about the sail-line (a 300 m spread between outermost streamers);
- a 25 m along-sail-line, signal spacing;
- a 400 m sail-line spacing;
- 50 m water depth;
- 2 lines sailed adjacent the chosen sail-line;
- an east-west orientation;
- a 2km grid about the central receiver location (point [0 0]).

The water depth was chosen to be representative of that from an area commonly fished for scallops. Five sail lines were used, so statistics per line and the cumulative or maximum levels reached considering one or all lines were calculated. The geometry of a modelled survey are shown on Figure 17 for one set of five sail lines where the sail line is directly over the receiver location (the sources are thus 50 m offset horizontally). Note that the air gun sources are each 50 m adjacent the sail line, so maximum exposure is reached when a sail line has a 50 m offset form the receiver (always at [0 0]). The model was set up using empirical fits given below and run with a series of sail-line offsets.

In order to model received levels, empirical curves of transmission for sound exposure level and maximum magnitude of ground roll were sourced for a large air gun array, as best as possible operating over sand in a similar water depth. From the seismic decay curves with range given in McCauley *et al*. (2016) we get a relationship of SEL with range using the mean of a 3090 and 3040 in^3 source operating over sand in 100-150 m water depth (each source gave a correlation when using a curve of the form below of r^2=0.99 and r^2=0.95 with similar values for each constant), defined by:

$$RL = \left[-23.33 * \log_{10}(R)\right] + \left[-0.003 * R\right] + 234.8$$

where *RL* is received SEL (dB re 1µPa^2·s), and *R* is slant range (direct range source to seabed receiver in m). These values were considered to apply to a 3065 in^3 source.

A relationship for the maximum magnitude of ground roll with range was derived using the same instruments as used here and a 3130 in^3 source operating in 40 m of water (RDM, unpublished data) as:

$$GR_a = [-11.51 * \log_{10}(R)] + [-0.0004 * R] + 36.9$$

where GR_a is the maximum magnitude of ground roll in dB values (dB re 1ms^{-2}) for each air gun signal and R is horizontal range (m). The measured data gave a correlation coefficient using this fit of r^2=0.85.

For comparison the 150 in^3 air gun measured here during the scallop experiments (45 and 150 in^3) gave a fitted curve for ground roll of:

$$GR_b = [-6.88 * \log_{10}(R)] + [-0.0021 * R] + 21.1$$

where GR_b is the maximum magnitude of ground roll in dB values (dB re 1ms^{-2}) for each air gun signal and R is horizontal range (m). The measured data gave a correlation coefficient using this fit of r^2=0.78.

Using these semi-empirical fits and the geometry including five sail lines so as to estimate cumulative exposures of multiple passes, the curves for closest sail line and: 1) estimated maximum magnitude of ground roll for any single signal (no cumulative effects considered for ground roll); 2) the maximum sound exposure level (SEL) experienced along any line; and 3) the cumulative SEL for all five lines, are shown on Figure 18. Note that when the model was run with only one sail-line, the cumulative SEL values shifted down by only a fraction of a dB. The x-axis given on Figure 18 is for the closest sail line to the receiver location, thus the other four lines in the calculations would have been adjacent this sail line and away from the receiver.

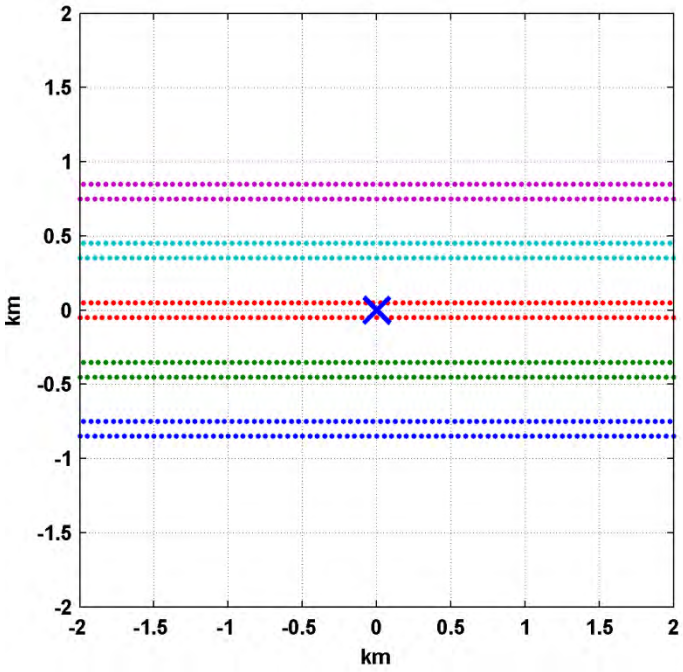


Figure 17: Spatial layout of hypothetical seismic survey lines used to estimate exposure. The start sail line offset here is 50 m, the receiver location is at the centre, and air gun signal locations are shown by the dots.

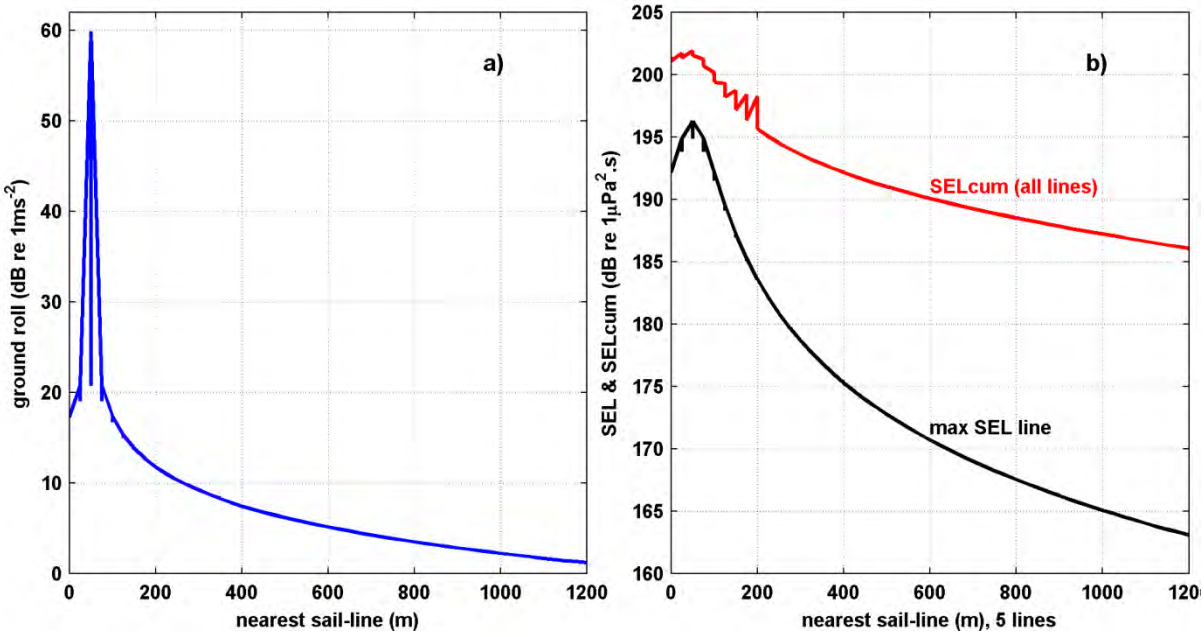


Figure 18: Estimated ground roll and sound exposure level of hypothetical seismic survey, showing: a) Estimated ground roll for a large commercial seismic source with nearest sail-line; b) For the nearest sail-line, maximum sound exposure level (SEL, black curve) for any sail line and cumulative SEL (red curve) for the five sail lines used in modelling.

Comparison ambient noise, lobster collection sites

Lobsters were collected at two sites for the 2014 experiments, off Crayfish Point Reserve, Taroona (on the western side of the Derwent River, a shipping route) and the remote southern Tasmanian coast (see Figure 5 for locations). Well after experiments had been conducted and physiological examinations made, we ascertained control lobsters from the different sites had different morphological damage to their statocyst systems (see Lobster section below). Control lobsters collected from the Taroona site had damaged statocyst organs prior to exposure in experiments, whereas those from the remote location did not. To assess what previous sound exposure history animals may have encountered, sea noise loggers were placed at the sites simultaneously for an extended period (data sets 3432 and 3433 as listed in Table 3), with overlapping samples taken between16-Jun-2015 to 16-Jul-2015. Sea noise loggers were set on the seabed with hydrophones lying on the reef structure.

The low frequency ambient noise differed between the sites as can be seen from 24 hour averaged sea noise spectra made on three consecutive days at each site which is shown in Figure 19. The sea noise spectra are similar above ~ 700 Hz between sites, where they are dominated by snapping shrimp noise, and appear to be similar at and below 10 Hz. But between 10 and 700 Hz the averaged spectra at Taroona was 5-10 dB higher than at the southern Tasmanian site. Sea noise at the Taroona site had: 1) incidences of relatively high noise levels associated with nearby ship passage; and 2) more or less continual low frequency tones possibly associated with localised pumping systems. This pump noise has increased the time averaged spectra shown in Figure 19 between 10-700 Hz. Examples of the pump noise are shown on Figure 20. The tones were intermittent at various levels and frequencies.

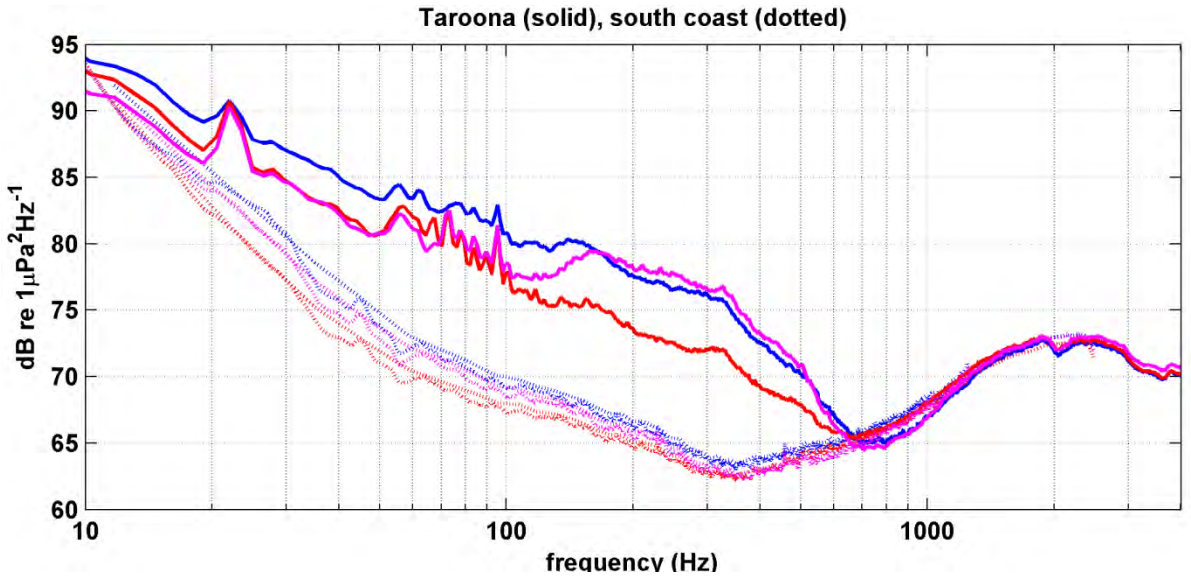


Figure 19. Averaged sea noise made from lobster collection sites across 24 hour periods over 9th (blue), 10th (red) and 11th (magenta) of July-2015 from Taroona (solid lines) and the southern Tasmanian coast (dotted curves).

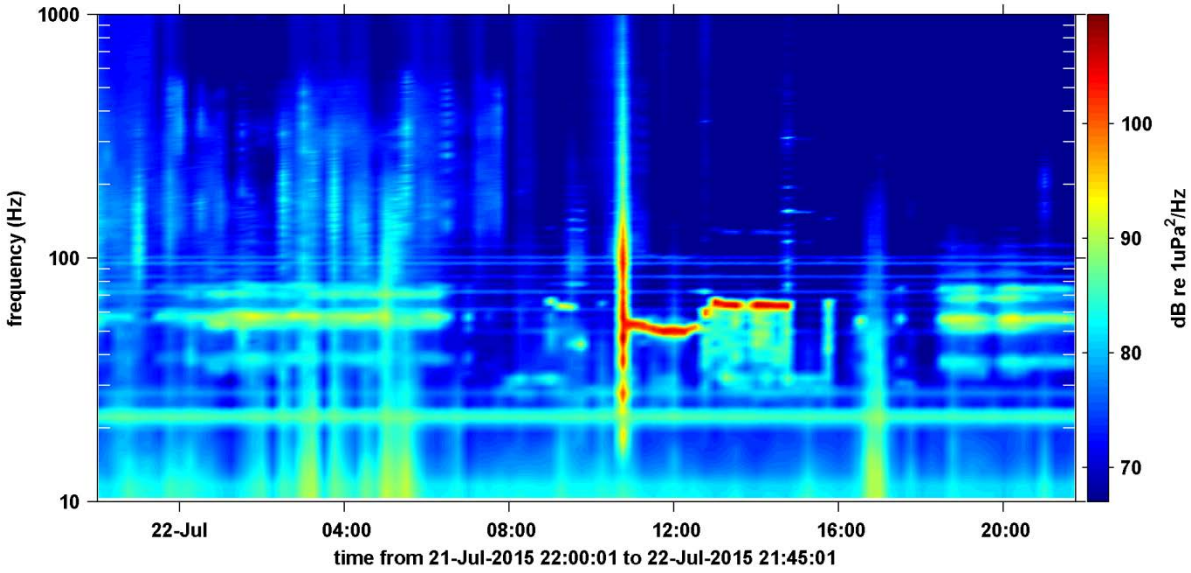


Figure 20. A 24 hour spectrogram of sea noise from the Crayfish Reserve, Taroona site (log frequency scale) showing tones associated with various continuous unidentified anthropogenic noise signals (horizontal bands of energy) and a vessel noise spike (at around 10:00 hours).

To highlight differences in ambient sea noise levels between the sites, broadband sea noise levels (across 8 Hz to 3 kHz) were calculated for the Crayfish Reserve, Taroona and South Coast sites in consecutive 9.6 s periods (6 kHz sample rate used, 0.73 Hz resolution, 8192 point FFT, seven averages) over 12.6 days which overlapped between the two sites. The normalised distribution of these broadband levels is shown on Figure 21. While both sites had similar maximum broadband levels reached (153 and 150 dB re 1µPa, Taroona and South Coast respectively), the Taroona site had more frequent higher level signals than the south coast site with the broadband level distribution skewed to the right.

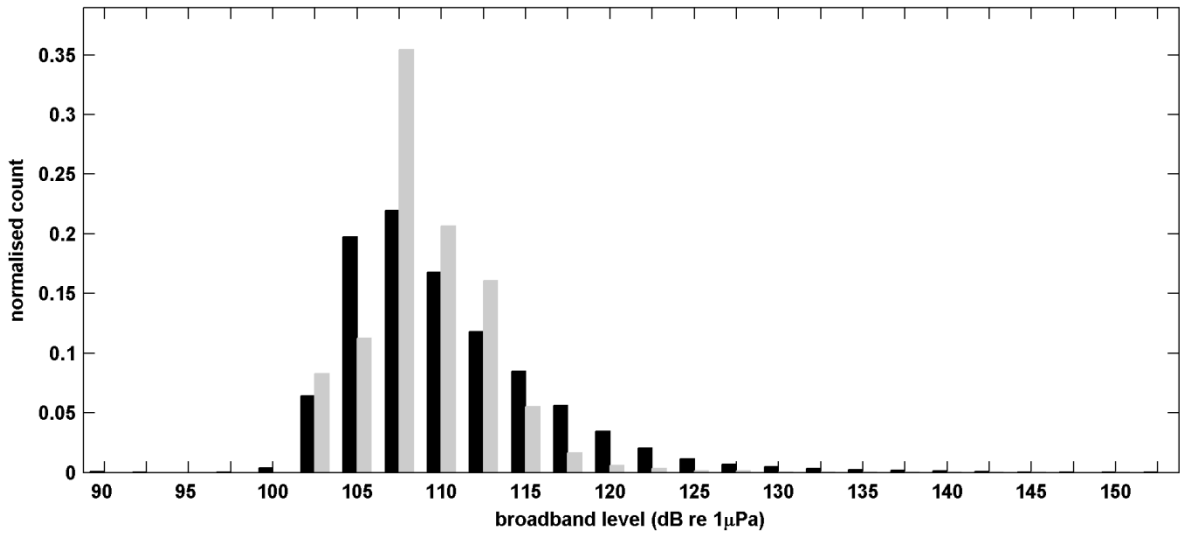


Figure 21. Normalised distribution of broadband ambient sea noise from the Crayfish Reserve site off Taroona (dark bars) and the southern coast (light grey bars). A 2.5 dB resolution was used but only the centre of every second point is shown.

Lobster Results

Air gun exposures

Estimates of sound exposure (SEL) and peak-peak level for each air gun signal were made at all lobster pots using empirical measures made in the field adjusted for air gun source levels (see General Results), which showed peak-to-peak source levels (at 1 m reference distance) of 223, 224 and 227 dB re 1µPa in the winter 2013, 45 in^3 standard pressure experiment; the winter 2014, 150 in^3 low pressure experiment; the winter 2014, 150 in^3 standard pressure experiment and the summer 2015, 150 in^3 standard pressure experiment, respectively, and SEL source levels for the same estimated at 200, 203, 205 dB re 1 µPa2·s respectively, with the 150 in^3 low pressure experiment functioning as a moderate exposure level relative to the lower intensity 45 in^3 and the higher intensity 150 in^3 standard experiments, thus increasing the spread of exposures.

Maximum exposure received at any pot was dependent on proximity to the air gun which was random amongst experiments, but the cumulative sound loading, or total dose of sound received per exposure, was lowest for the winter 2013, 45 in^3 standard pressure experiment, intermediate for the winter 2014, 150 in^3 low pressure experiment and highest for the two 150 in^3 standard pressure experiments (winter 2014 and summer 2015) as given by the number of signals which exceeded set thresholds and the maximum or median cumulative sound exposure SEL_{cum} (Table 5). The maximum and median cumulative sound exposure level estimated in the three experimental regimes were 192 and 191 for the winter 2013, 45 in^3 standard pressure experiment, 193 and 192 for the winter 2014, 150 in^3 low pressure experiment and 199 and 197 dB re 1µPa^2·s for the two 150 in^3 standard pressure experiments, while the maximum number of shots amongst pots exceeding 180 dB re 1µPa^2·s differed substantially, at 3, 7 and 25 for the 45 in^3, 150 in^3 low pressure and 150 in^3 standard pressure exposures, respectively (Table 5).

Mortality

No mortalities were recorded in either control or exposed treatments at any point in any of the four experiments comprising this study.

Reflex behaviour

In the winter 2013, 45 in^3 standard pressure experiment, sample time was found to be a significant factor in relative tail gape ($F(3,95)=14.44$, $P<0.001$), with lobsters at day 120 showing a reduction in relative tail gape of 19% of that of lobsters sampled at days 0, 24% of that of lobsters sampled at day 2 and 27% of that of lobsters sampled at 14, indicating a greater capacity for tail flexion at the later sample point (Fig. 22A). A similar result was found in the 2014 150 in^3 low pressure experiment (Fig. 22B), as time was again a significant factor ($F(4,71)=5.956$, $P<0.001$) as lobsters sampled on day 120 had a 39% reduction in tail gape compared to lobsters sampled on day 0 and 33% compared to lobsters sampled on day

14. In the summer 2015, 150 in^3 standard pressure experiment (Fig. 22C), both exposure ($F(1,50)=22.182$, $P<0.001$) and time ($F(1,50)=15.887$, $P<0.001$) were significant factors, though there was no significant interaction. Exposure resulted in a 32% increase in tail gape, indicating a reduced ability to maintain tail flexion. Lobsters sampled at day 14 post-exposure also demonstrated a reduced capacity for tail flexion, with a tail gape 23% greater than lobsters sampled at day 2. In the winter 2014, 150 in^3 standard pressure experiment with male lobsters (Fig. 22D), which were sampled at days 0, 2 and 14 post-exposure, there was no significant response to either exposure or across sample times. Berried females sampled at days 0-14 (Fig. 22E), sampled on the same schedule, showed a significant response to sample time ($F(2,38)=14.014$, $P<0.001$), with lobsters sampled at day 0 showing a significantly greater relative tail gape than when sampled at days 2 or 14. Relative tail gape did not differ in the berried females sampled at day 120.

Comparisons of righting reflex time in the winter 2013 45 in^3 standard pressure experiment (Fig. 23A) showed that exposure was a significant factor ($F(1,95)=44.87$, $P<0.001$), as exposed lobsters showed a 157% increase in righting time over that of control animals. In the winter 2014 150 in^3 low pressure (Fig. 23B) experiment, again, only exposure was a significant factor ($F(1,71)=70.417$, $P<0.001$), with exposed lobsters taking 120% longer to right than control lobsters. In the summer 2015 150 in^3 standard pressure experiment (Fig. 23C), exposure was again the only significant factor ($F(1,50)=8.557$, $P=0.005$), as exposed lobsters required 80% more time to right than controls. In the winter 2014 150 in^3 standard pressure experiment, neither sample day nor treatment were found to have a significant effect on righting time in males (Fig. 23D), berried females sampled at days 0-14 post-exposure (Fig. 23E) or berried females sampled at day 120 post-exposure (Fig. 23).

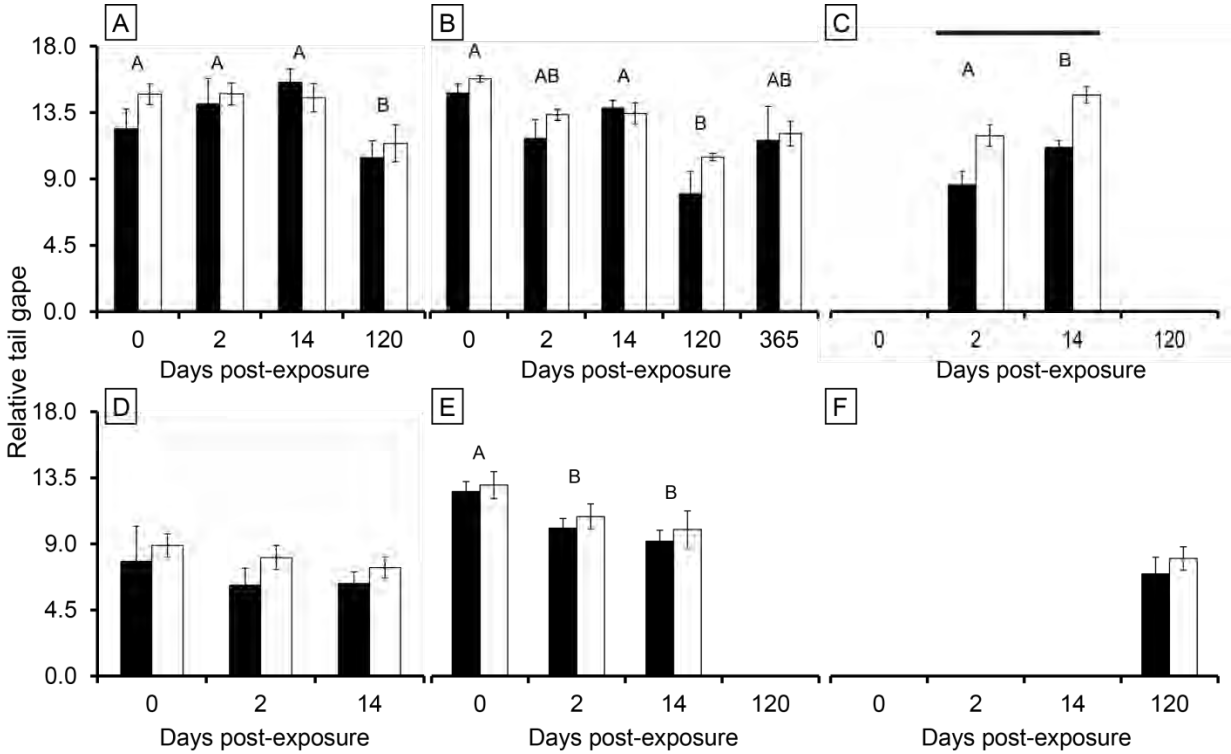


Figure 22. Mean (± SEM) relative tail gape, measured as percent carapace length, in lobsters from control (black) and exposed (white) treatments at days 0, 2, 14, 120 and 365 from the a) winter 2013, 45 in^3 standard pressure air gun experiment (control treatment day 0 n=11, day 2 n=10, day 14 n=10, day 120 n=11; exposed treatment day 0 n=10, day 2 n=10, day 14 n=11, day 120 n=10), b) winter 2014, 150 in^3 low pressure air gun experiment (control treatment day 0 n=9, day 2 n=9, day 14 n=8, day 120 n=8 day 365 n=7; exposed treatment day 0 n=8, day 2 n=8, day 14 n=8, day 120 n=7, day 365 n=10) , c) summer 2015, 150 in^3 standard pressure air gun experiment (control treatment day 2 n=12, day 14 n=14; exposed treatment day 2 n=15, day 14 n=13), d) winter 2014, 150 in^3 standard pressure air gun experiment males (control treatment n=11, same individuals sampled at days 0, 2 and 14; exposed treatment n=10, same individuals sampled at days 0, 2 and 14), e) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at days 0-14 post-exposure (control treatment n=11, same individuals sampled at day 0, 2, 14; exposed treatment n=10, same individuals sampled at days 0, 2, 14) and f) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at day 120 post-exposure (control treatment n=11; exposed treatment n=10). For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (a, b, c) or mixed-design ANOVAs (d, e). Significant differences in response to exposure as determined by Welch two-sample t-test (f) are also indicated by a horizontal bar.

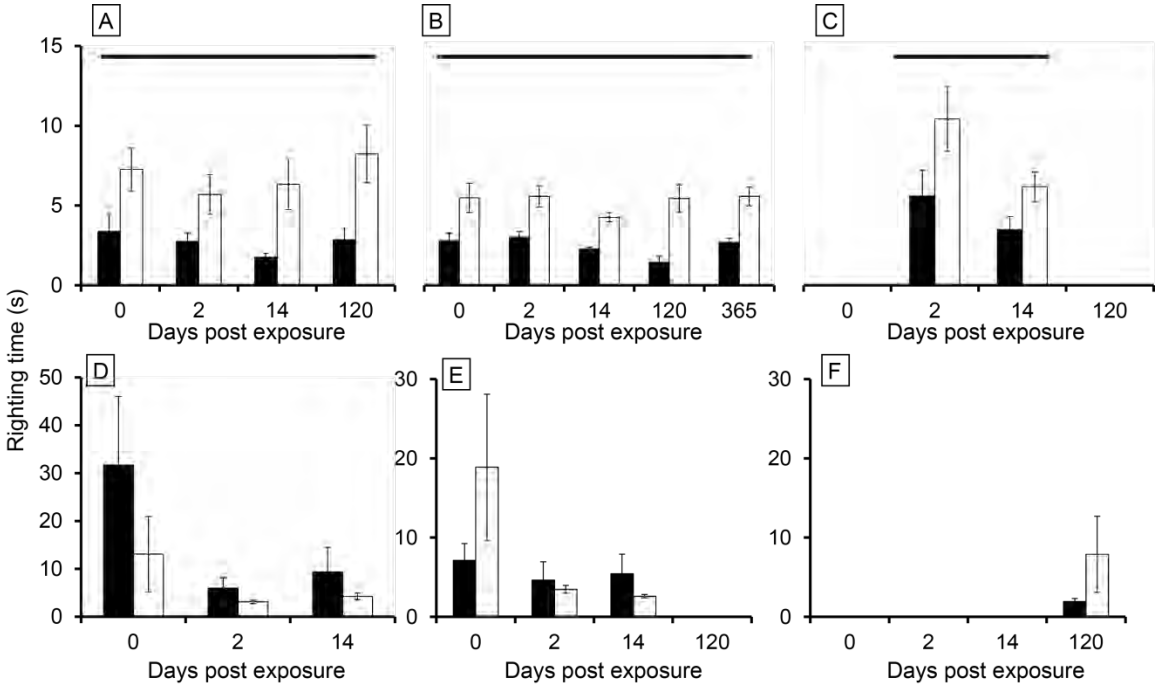


Figure 23. Mean (± SEM) righting time, measured in seconds, in lobsters from control (black) and exposed (white) treatments at days 0, 2, 14, 120 and 365 from the a) winter 2013, 45 in^3 standard pressure air gun experiment (control treatment day 0 n=11, day 2 n=10, day 14 n=10, day 120 n=11; exposed treatment day 0 n=10, day 2 n=10, day 14 n=11, day 120 n=10), b) winter 2014, 150 in^3 low pressure air gun experiment (control treatment day 0 n=9, day 2 n=9, day 14 n=8, day 120 n=8 day 365 n=7; exposed treatment day 0 n=8, day 2 n=8, day 14 n=8, day 120 n=7, day 365 n=10) , c) summer 2015, 150 in^3 standard pressure air gun experiment (control treatment day 2 n=12, day 14 n=14; exposed treatment day 2 n=15, day 14 n=13), d) winter 2014, 150 in^3 standard pressure air gun experiment males (control treatment n=11, same individuals sampled at days 0, 2 and 14; exposed treatment n=10, same individuals sampled at days 0, 2 and 14), e) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at days 0-14 post-exposure (control treatment n=11, same individuals sampled at day 0, 2, 14; exposed treatment n=10, same individuals sampled at days 0, 2, 14) and f) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at day 120 post-exposure (control treatment n=11; exposed treatment n=10). For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (a, b, c) or mixed-design ANOVAs (d, e). Significant differences in response to exposure as determined by Welch two-sample t-test (f) are also indicted by a horizontal bar.

Statocyst analysis

The rock lobster statocyst was roughly reniform (Fig. 24), with four zones of hair cells identified (Figs. 25, 26) based on subtle differences in the arrangement and density of hairs in each zone. Zone 1 was located at the opening of the statocyst, and consisted of a dense grouping of long hairs that extended toward the 'corpus,' or body, of the statocyst with the hairs oriented towards the statocyst opening. Zone 2 was a large region consisting of a diffuse arrangement of hairs that occurred in pairs in the area more proximal to Zone 1 and in triplets in areas more distal. The hairs were roughly aligned in rows running perpendicular to the orientation of Zone 1 hairs. Hair cells in Zone 3 were loosely arranged within a band running along the outside curve of the corpus of the statocyst. Zone 4 continued from Zone 3 into the smaller curve, or "end", of the kidney shaped statocyst, and was differentiated by a slight but consistent gap in the hairs between Zones 3 and 4, as well as a somewhat reduced density of hair cells, based on qualitative observation. This delineation between zones 3 and 4 was clear and consistent, so it was not tested statistically. Zones 3 and 4 were found to be the primary site of contact between hair cells and statoconia (Fig. 27), the assemblage of small, dense particles (i.e. sand, sponge spicules, shell grit, etc.) that act on the sensory hairs. Damage to the hair cells presented as the loss of the hair, leaving only a pore (Figs. 28, 29, 30)

Figures 31 and 32 and Table 8 show the proportion of hair damage across all treatments, controls and zones. This clearly indicates a higher level of damage in zones 3 and 4 in exposed lobsters in three of the treatments. This also suggests that lobsters in the control groups had differing levels of damage, which is possible due to different sources of experimental animals for the four experiments.

The mixed effects logistic regression found that the control groups were significantly different except the 2013, 45 in^3 standard pressure and winter 2014, 150 in^3 low pressure experiments. Consequently, the controls cannot be pooled and it is necessary to consider the difference between each treatment and its control (see results in Tables 9 and 10).

The relationship between the proportion of damaged hairs and lobster size (mass and carapace length) was not statistically significant (respectively $p=0.16$ and $p=0.13$). Comparisons between control groups and exposed groups revealed that all exposed groups had a higher proportion of damaged hairs (Table 9), which did not differ significantly across exposure treatments. However, as the control groups had significant differences in hair damage it may be more appropriate to consider the inferred additional hair damage due to the exposure. This effect was statistically significant for three exposures (Table 10) -- it was highest for the winter 2013, 45 in^3 standard pressure experiment exposed treatment, second highest (and equivalent) for the winter 2014, 150 in^3 low pressure experiment and summer 2015 150 in^3 standard pressure experiment exposed treatments and not significant for the winter 2014, 150 in^3 standard pressure experiment exposed treatment. In the latter case the results from the control group suggest that the experimental lobsters had a high level of pre-existing damage when compared to other years.

Lobster righting time (Fig. 33) was explored using a generalised linear model. A Box-Cox analysis indicated a log transform was suitable. The proportion of damaged hair was not significant ($p=0.12$) once the treatment was included in the model. This indicates that the relationship between righting time and the exposure event is stronger than the relationship between with the level of damage that event caused.

Larger lobsters had longer righting times ($p<0.001$) and the interaction between the treatment and exposure was significant ($p<0.001$) indicating that the exposures impacted the righting times in different ways. Interestingly out of the exposed groups, the 2014 High Pressure exposed group had the fastest righting times (Table 11), possibly indicating that pre-existing exposure to damaging sound levels had allowed the individuals to adapt / compensate for subsequent exposures.

Figure 24. Intact lobster statocyst with antennule cuticle removed, showing the reniform shape of the statocyst. The opening of the statocyst, located on the dorsal side of the antennule, is to the left and the arrow points anteriorly.

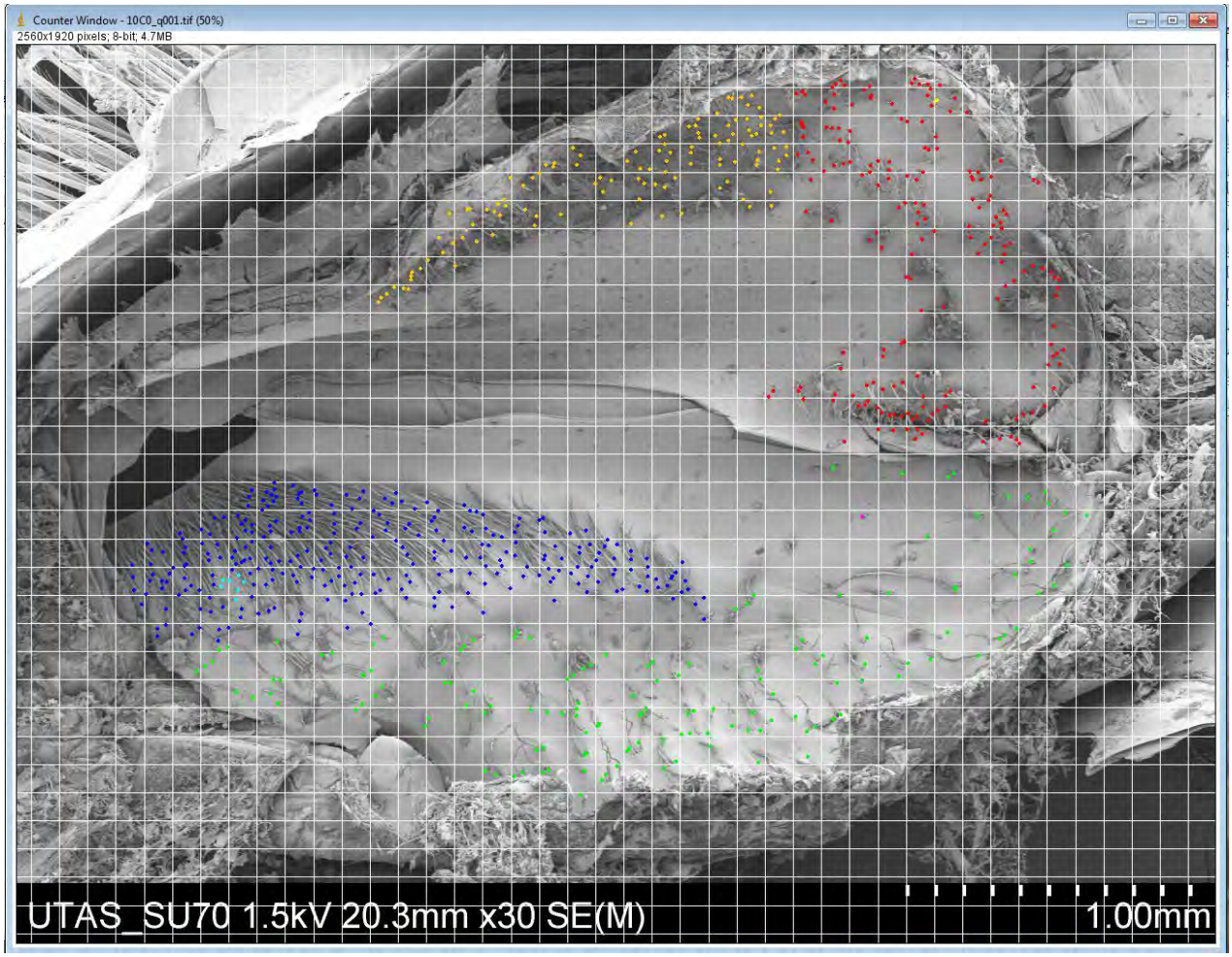


Figure 25. Statocyst image analysis. Images of statocysts obtained using scanning electron microscopy were analysed using a 0.01 mm grid imposed on the image and damaged and undamaged hair cells were counted. Zone 1 is indicated in blue, zone 2 in green, zone 3 in gold and zone 4 in red; see results section for description of zones. Damaged hair cells occurring within the same box as structural damage to the statocyst capsule were excluded from counting, whereas healthy hairs were included.

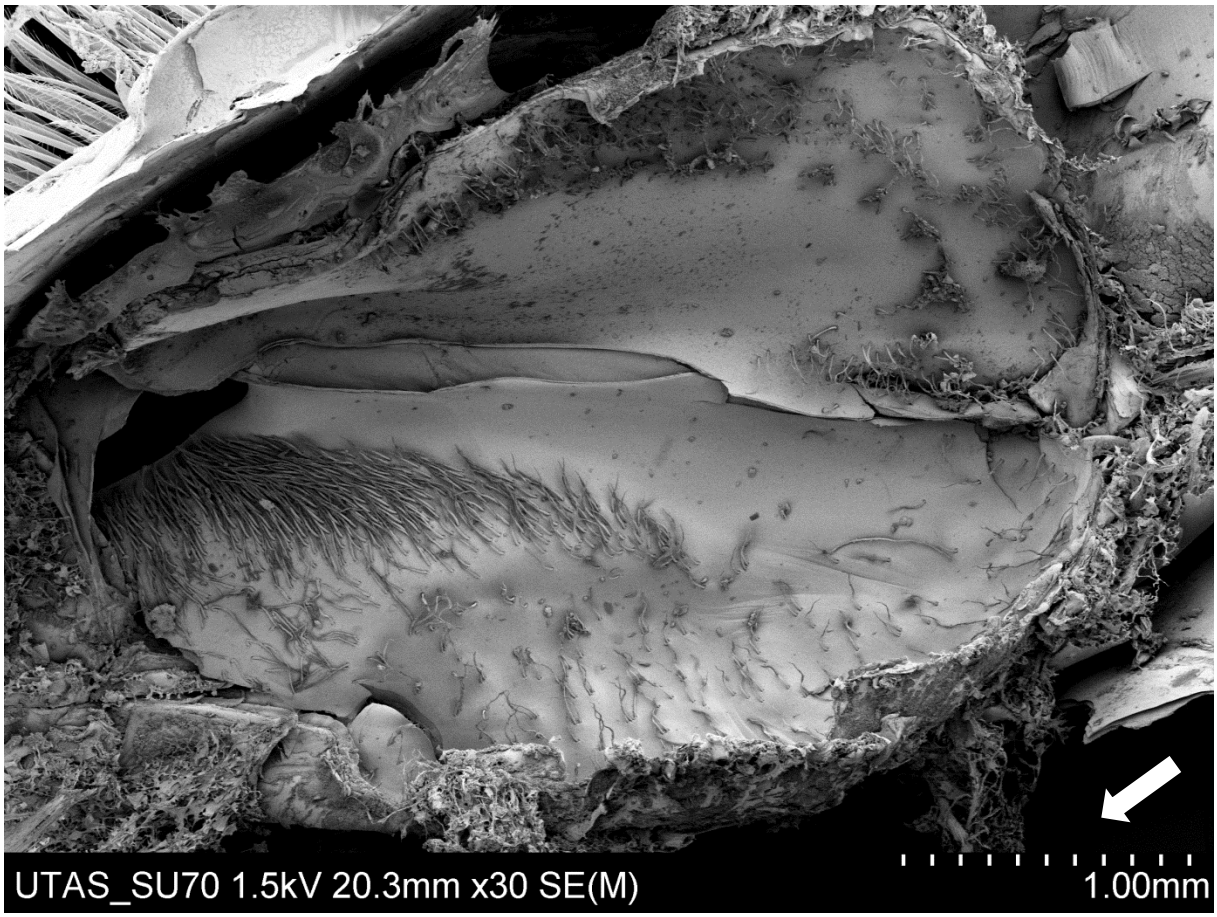


Figure 26. Lobster statocyst following dissection. Statocysts were split longitudinally along the long bends to expose the hair cells. Statoconia and mucus have been removed using jets of 70% ethanol. Arrow points anteriorly.

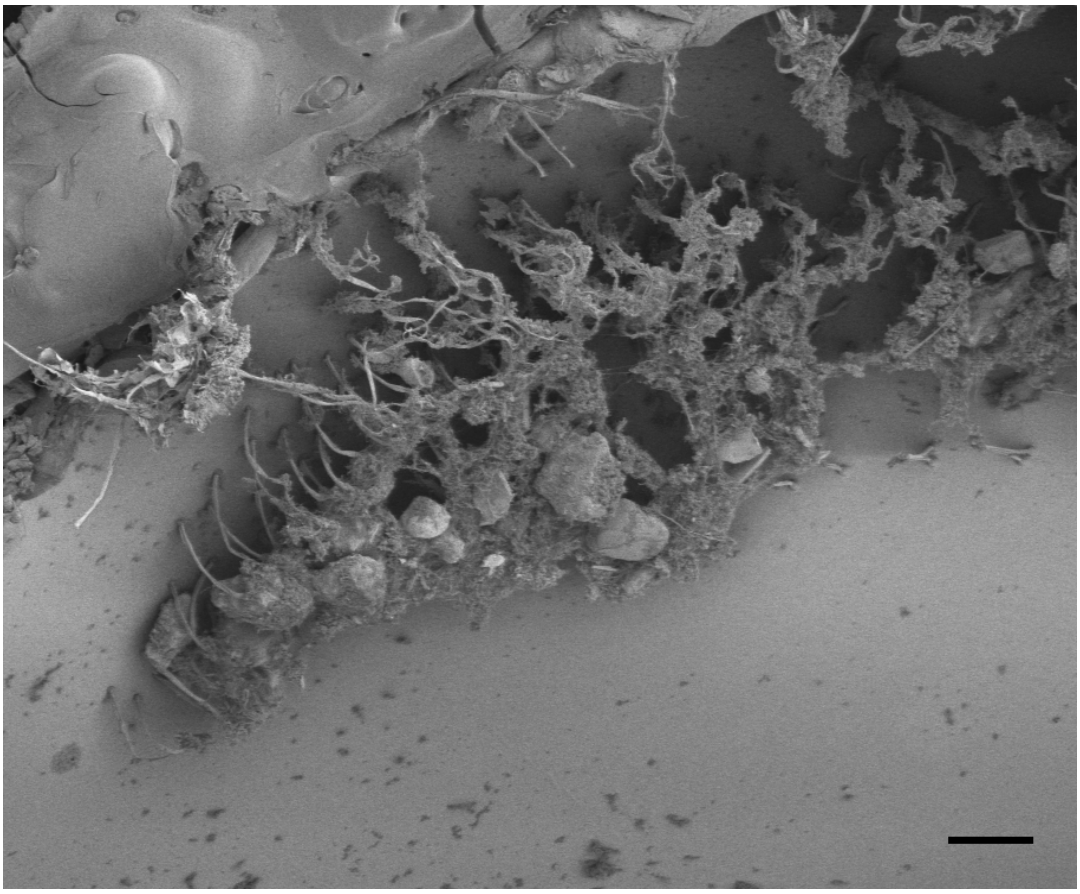


Figure 27. Statoconia adhering to hairs within lobster statocyst. Scale bar indicates 0.1 mm.

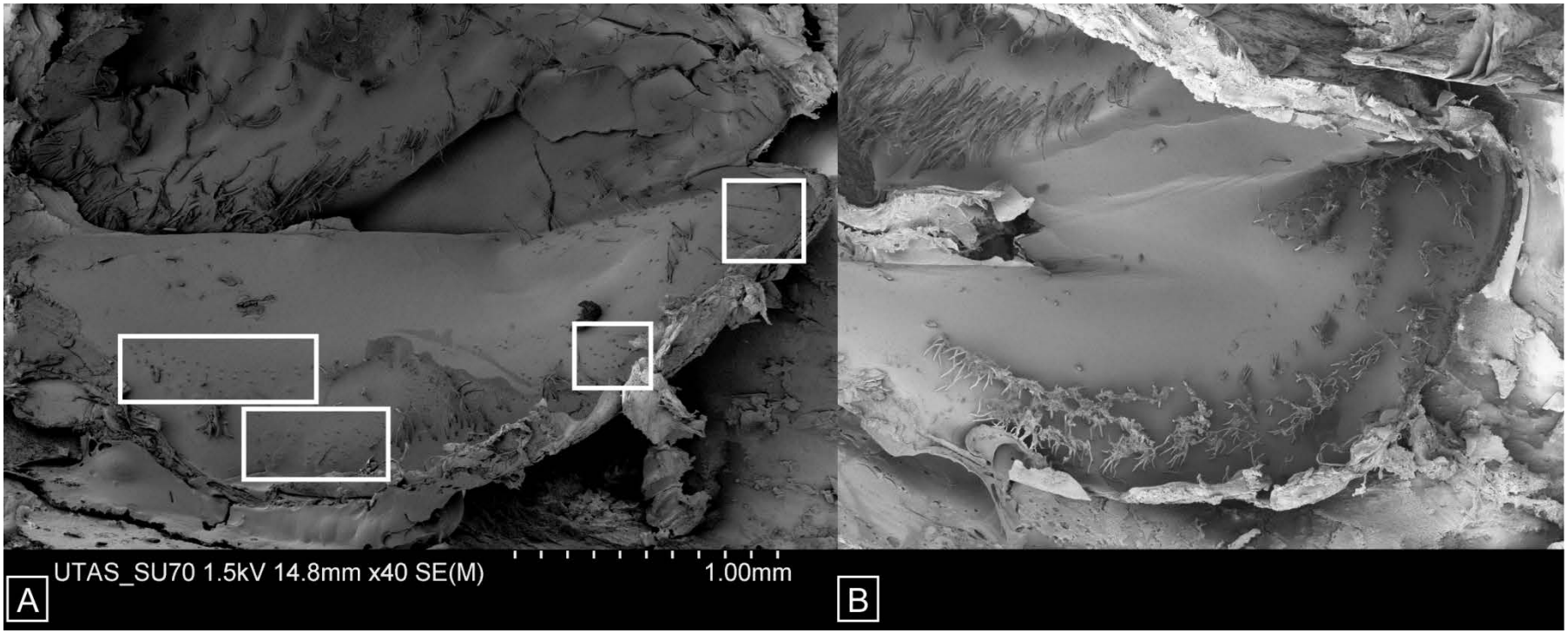


Figure 28. Image of damaged fields of hair cells in a statocyst from an exposed lobster (A) and the same fields, undamaged, in the statocyst of a control lobster (B). Fields of missing hair cells are indicated by white boxes, though numerous other areas of damage are visible.

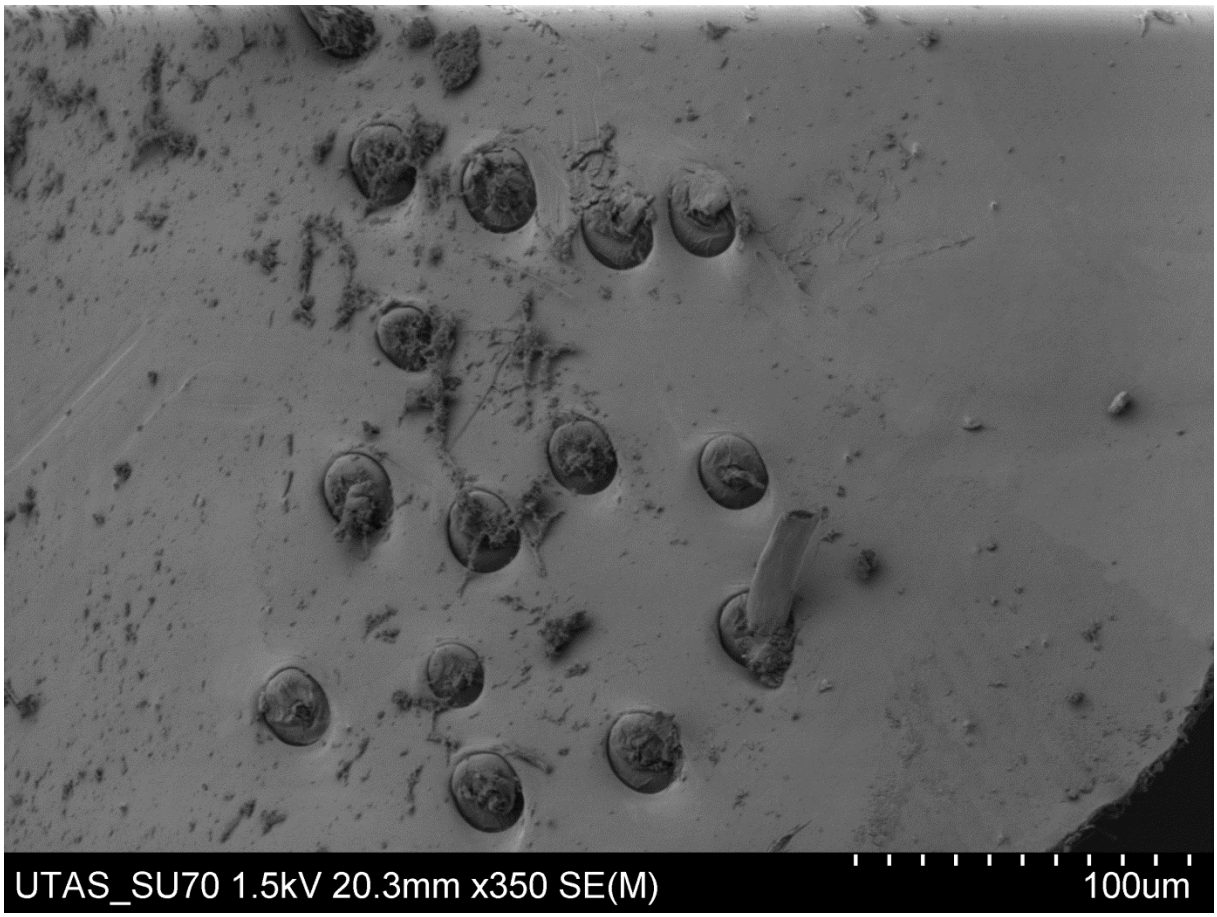


Figure 29. Close up of damaged hair cells. Hairs were detached at the casque, the flexible joint located where the hair cell emerges from the pore.

Figure 30. Close up of damaged hair cells. Note that intact hair cells are immediately adjacent, demonstrating the haphazard distribution of damage common throughout samples.

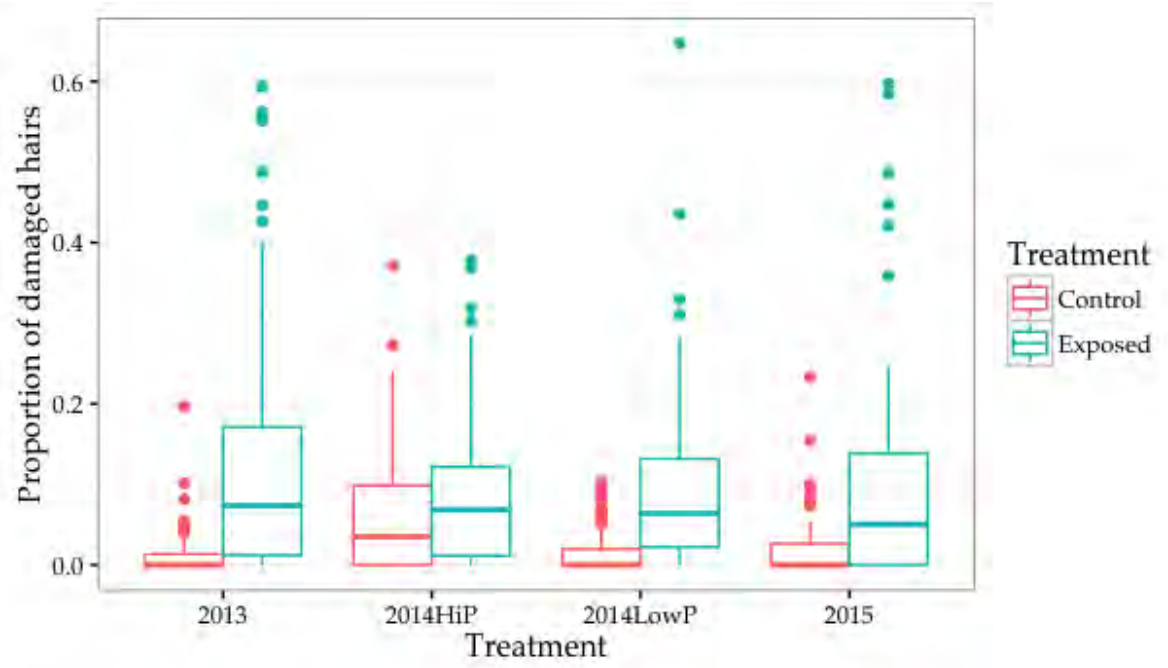


Figure 31. Proportion of damaged hairs from the statocysts of lobsters from control (red) and exposed (cyan) treatments from the winter 2013, 45 in^3 standard pressure air gun experiment (control treatment n=35; exposed treatment n=38), winter 2014, 150 in^3 low pressure air gun experiment (control treatment n=38; exposed treatment n=39), winter 2014, 150 in^3 standard pressure air gun experiment (control treatment n=27; exposed treatment n=26), and summer 2015, 150 in^3 standard pressure air gun experiment (control treatment n=20; exposed treatment n=20). Each point corresponds to one zone in an individual lobster. The box extends from the 25th to the 75th percentile, and the vertical lines (whisker) from the lowest/highest values that are within 1.5 inter-quartile ranges of the 25th/27th percentile. Remaining outliers are shown by dots.

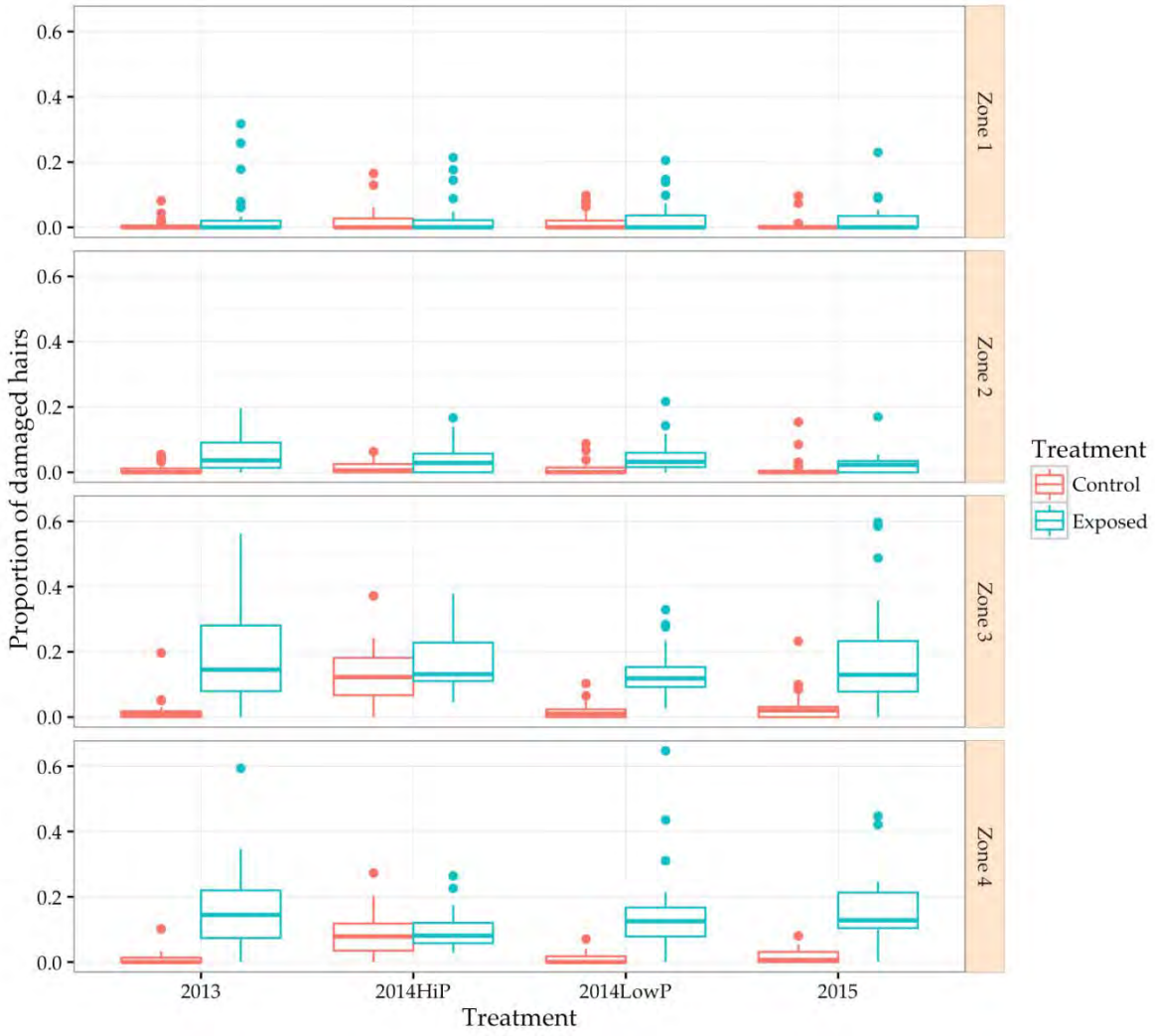


Figure 32. The proportion of damaged hairs by zone in the statocysts of lobsters from control (red) and exposed (cyan) treatments from the winter 2013, 45 in^3 standard pressure air gun experiment (control treatment n=35; exposed treatment n=38), winter 2014, 150 in^3 low pressure air gun experiment (control treatment n=38; exposed treatment n=39), winter 2014, 150 in^3 standard pressure air gun experiment (control treatment n=27; exposed treatment n=26), and summer 2015, 150 in^3 standard pressure air gun experiment (control treatment n=20; exposed treatment n=20). Each point corresponds to an individual lobster.

Table 8. The proportion of undamaged hairs from lobster statocysts by experiment and treatment (control or exposed) from the winter 2013, 45 in^3 standard pressure air gun experiment (control treatment n=35; exposed treatment n=38), winter 2014, 150 in^3 low pressure air gun experiment (control treatment n=38; exposed treatment n=39), winter 2014, 150 in^3 standard pressure air gun experiment (control treatment n=27; exposed treatment n=26), and summer 2015, 150 in^3 standard pressure air gun experiment (control treatment n=20; exposed treatment n=20).. The means and standard devations are taken across all lobsters and zones.

	Control		Exposed	
Experiment	Mean	SD	Mean	SD
Winter 2013, 45 in^3 standard pressure	0.99	0.02	0.89	0.13
Winter 2014, 150 in^3 standard pressure	0.94	0.07	0.91	0.09
Winter 2014, 150 in^3 low pressure	0.99	0.02	0.91	0.09
Summer 2015, 150 in^3 standard pressure	0.98	0.04	0.90	0.13

Table 9. Odds ratios from the logistic regression model used to analyse damage to statocysts of lobsters. These give the rate of damage relative to the reference category (Zone 1 for the Zone factors, 2013 Control for the Experiments). For example, the level of hair damage in the 2013 Exposed treatment was 14.5 times the 2013 Control treatment (reference category). Note that all Exposures had a similar (not significantly different) level of hair damage.

		95% CI	
Factor	Estimate	Lower	Upper
Zone 2	1.3	1.1	1.4
Zone 3	6.2	5.6	6.8
Zone 4	4.9	4.4	5.4
Winter 2014, 150 in^3 standard pressure Control	7.7	5.1	11.7
Winter 2014, 150 in^3 low pressure Control	1.4	0.9	2.1
Summer 2015, 150 in^3 standard pressure Control	1.8	1.1	2.8
Winter 2013, 45 in^3 standard pressure Exposed	14.5	9.9	21.4
Winter 2014, 150 in^3 standard pressure Exposed	10.8	7.1	16.4
Winter 2014, 150 in^3 standard pressure Exposed	10.5	7.2	15.5
Summer 2015, 150 in^3 standard pressure Exposed	11.2	7.2	17.6

Table 10. Odds ratios from the logistic regression model used to analyse statocyst damage in lobsters, expressed to give the increase in hair damage from each exposure relative to the associated control. Additional hair damage was highest for the winter 2013, 45 in^3 standard pressure experiment, with 14.5 times the level in the winter 2013, 45 in^3 standard pressure control. It was lowest in the winter 2014, 150 in^3 standard pressure experiment, where relative to the winter 2015, 150 in^3 standard pressure control treatment, there was no significant difference (a value of 1 indicates no change and this is spanned by the confidence interval).

		95% CI	
Treatment	Estimate	Lower	Upper
Winter 2013, 45 in^3 standard pressure	14.5	9.9	21.4
Winter 2014, 150 in^3 standard pressure	1.4	0.9	2.1
Winter 2014, 150 in^3 low pressure	7.5	5.2	10.8
Summer 2015, 150 in^3 standard pressure	6.4	3.9	10.5

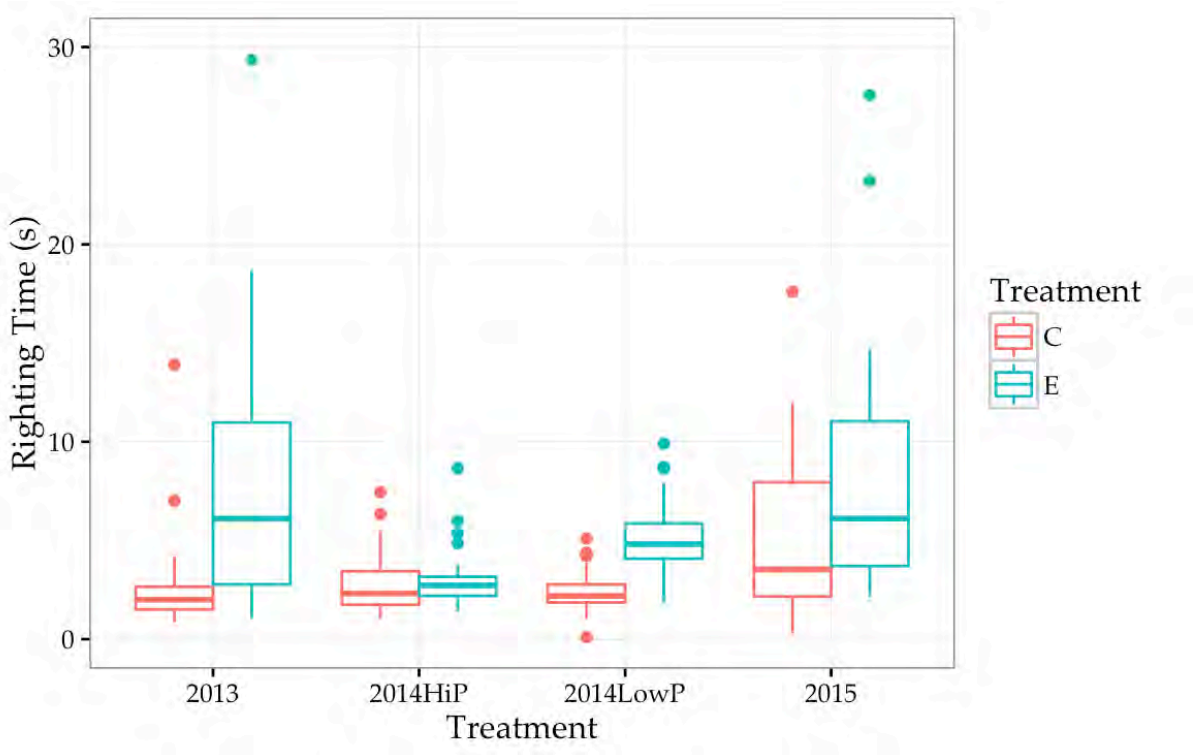


Figure 33. Righting time by treatment and experiment for lobsters in the winter 2013, 45 in^3 standard pressure air gun experiment (control treatment n=35; exposed treatment n=38), winter 2014, 150 in^3 low pressure air gun experiment (control treatment n=38; exposed treatment n=39), winter 2014, 150 in^3 standard pressure air gun experiment (control treatment n=27; exposed treatment n=26), and summer 2015, 150 in^3 standard pressure air gun experiment (control treatment n=20; exposed treatment n=20). For clarity, four outliers (two exposed, two control) with >30s righting time are not shown.

Table 11. The effect of exposure on the righting time relative of lobsters relative to the control treatment from the same experiment. Numbers are relative to the associated control, e.g. lobsters from the winter 2014, 150 in^3 low pressure exposed treatment had a righting time 2.2 times longer than the winter 2014, 150 in^3 low pressure control treatment.

		95% CI	
Treatment	Estimate	Lower	Upper
2013	2.6	2.2	3.1
2014 High P	1.1	0.9	1.3
2014 Low P	2.2	1.9	2.6
2015	1.7	1.4	2.1

Haemolymph

Analysis of total haemocyte counts (THC) in the winter 2013 45 in^3 standard pressure experiment (Fig. 34A) showed that exposure was a significant factor ($F(1,71)=11.974$, $P<0.001$), with exposed lobsters showing a 23% reduction in haemocytes per ml of haemolymph than control lobsters over all sample points. In the winter 2014 150 in^3 low pressure experiment (Fig. 34B), analysis showed a significant interaction between exposure and time ($F(4,68)=7.601$, $P<0.001$). Whereas both control and exposed treatments had similar THC levels at 0, 2 and 14 days post-exposure, at day 120, the exposed treatment showed a significantly reduced THC level compared to that of control treatments at days 0, 2, 14, 120 and 365 and exposed treatments at days 0, 2, 14 and 120. Compared to controls sampled at the same day, the day 120 exposed lobsters showed a 54% lower total haemocyte count. At the 365 day sample point, the opposite response was observed, as exposed lobsters showed an increase in THC to a level significantly greater than that of both treatments at all sample points, which was double that of the control treatment at the same sample point. In the summer 2015 150 in^3 standard pressure experiment (Fig. 34C), exposure was the only significant factor ($F(1,48)=14.046$, $P<0.001$), as exposed lobsters were found to have THC levels 41% lower than control lobsters.

In the winter 2014 150 in^3 standard pressure experiment, the THC of males (Fig. 34D) showed no effect from sample time, however, exposure was found to result in a significant ($F(1,17)=8.426$, $P=0.01$) 30% reduction in THC relative to that of control lobsters across the two sample days than the exposed treatment. A similar result was found in the females sampled at days 0-14 post-exposure (Fig. 34E), with sample time showing no effect and exposure showing a significant effect ($F(1,18)=6.197$, $P=0.02$), with exposure found to result in a 29% decrease in haemocytes compared to the control treatment. Females sampled at 120 days post-exposure also showed a significant difference between treatments at the lone 120

day sample point ($t(13.22)=5.83$, $P<0.001$), with exposed lobsters showing a reduction of 60% compared to controls.

Haemolymph pH showed a significant response to sample time in the winter 2013 45 in^3 standard pressure experiment (Fig. 35A) ($F(3,95)=15.006$, $P<0.001$), with samples taken at day 0 showing a significantly higher pH than samples at days 14 or 120 post-exposure. Sample time was also a significant factor in the winter 2014 150 in^3 low pressure experiment ($F(4,70)=13.228$, $P<0.001$), as pH was significantly higher in lobsters sampled at day 0 relative to day 2 and day 120, significantly higher at day 14 relative to day 2 and day 120 and significantly higher at day 365 than at day 0, day 2 and day 120. In the summer 2015 150 in^3 standard pressure experiment, time was again found to be a significant factor in haemolymph pH ($F(1,50)=34.287$, $P<0.001$), as lobsters sampled at day 14 had a pH lower than that of lobsters sampled at day 0. In the winter 2014 150 in^3 standard pressure experiment, males (Fig. 35D) were found to demonstrate a significant response in haemolymph pH to sample time ($F(1,19)=51.722$, $P<0.001$), with an increase from day 2 to day 14 of. Females sampled at 2 and 14 days post-exposure (Fig. 35E) also showed a significant response to sample time ($F(1,19)=6.678$, $P=0.02$), with an increase between days 2 and 14. No difference in haemolymph pH was found between treatments in females sampled at day 120 post-exposure.

Refractive index showed a significant ($F(3,95)=30.895$, $P<0.001$) response to sample time in the winter 2013 45 in^3 standard pressure (Fig. 36A), as day 120 lobsters had a RI approximately 60% higher than that of day 0, day 2 and day 14 lobsters. In the winter 2014 150 in^3 low pressure experiment, (Fig. 36B) refractive index showed a significant interaction between exposure and sample time ($F(4,70)=3.131$, $P=0.02$). There were no differences observed between the two treatments at days 0, 2 or 14, though day 0 lobsters were found to differ significantly to day 2 or 14 lobsters, with day 0 lobsters found to have RI values 5% and 9% lower, respectively. At day 120, RI increased by >70% relative to days 0, 2 and 14, with control lobsters demonstrating a 25% greater RI than exposed lobsters, a difference which was also significant. At day 365, both treatments showed a significant reduction in RI compared to day 120, but still at a level significantly greater than the first three sample points. Control lobsters at this sample point had an RI 15% greater than exposed lobsters, a difference that was again significant. In the summer 2015 150 in^3 standard pressure experiment (Fig. 36C), neither exposure nor sample time were a significant factor on refractive index and there was no interaction between the two factors. Likewise, in the winter 2014 150 in^3 standard pressure experiment, refractive index showed no response to either exposure or sample time in males, females sampled at days 2 and 14 post-exposure or females sampled 120 days post-exposure.

Analysis of haemolymph biochemistry from lobsters in the winter 2014, 150 in^3 low pressure experiment showed no significant difference between control and exposed groups in the 23 parameters at the various post-exposure times tested (Table 12). Many haemolymph parameters such as calcium, phosphorus, total protein, triglycerides, cholesterol, glucose, lactate and glutamate dehydrogenase were significantly higher at day 120 than at the beginning of the experiment (day 0, 2, and 14). There was also a significant decrease in the Na:K ratio with time for both control and exposed groups (Table 12).

From the lobsters in the winter 2014, 150 in^3 standard pressure experiment, none of the 23 haemolymph biochemistry parameters showed any significant difference between control and exposed groups (Table 13). There was no interaction with sex or day post-exposure in female lobsters (Table 13). Male lobsters showed significantly higher levels of Brix index, magnesium, calcium, phosphorus, total protein, triglyceride, cholesterol, and glucose than female lobsters at day 2 (Table 13). Female lobsters at day 120 showed significantly lower pH and magnesium, and significantly higher Brix index, calcium, phosphorus, total protein, triglycerides, cholesterol, glucose, lactate, uric acid, LIP, AST, GGT and GDH than females lobsters at day 2 (Table 13).

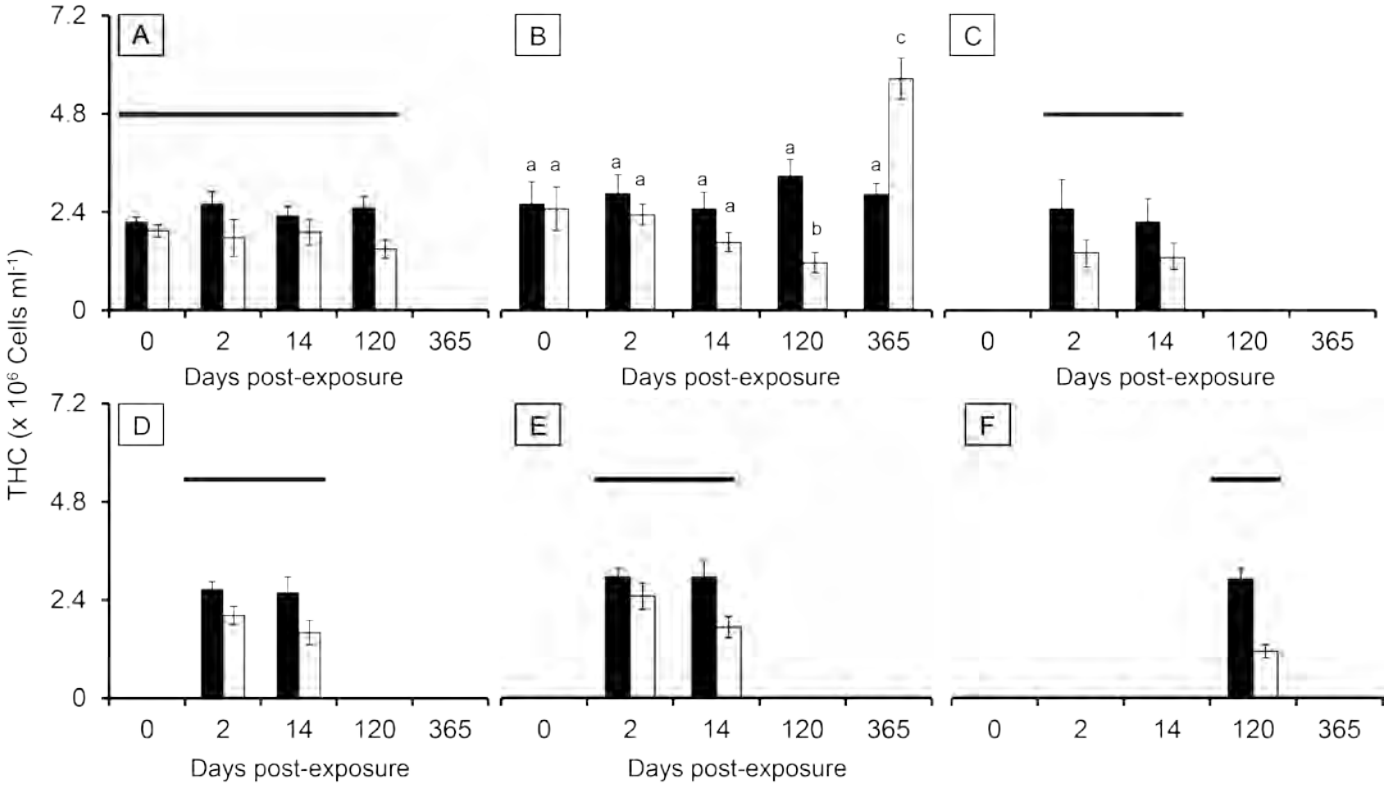


Figure 34. Mean (± SEM) total haemocyte count (THC) in lobsters from control (black) and exposed (white) treatments at days 0, 2, 14, 120 and 365 from the a) winter 2013, 45 in^3 standard pressure air gun experiment (control treatment day 0 n=11, day 2 n=10, day 14 n=10, day 120 n=11; exposed treatment day 0 n=10, day 2 n=10, day 14 n=11, day 120 n=10), b) winter 2014, 150 in^3 low pressure air gun experiment (control treatment day 0 n=9, day 2 n=9, day 14 n=8, day 120 n=8 day 365 n=7; exposed treatment day 0 n=8, day 2 n=8, day 14 n=8, day 120 n=7, day 365 n=10) , c) summer 2015, 150 in^3 standard pressure air gun experiment (control treatment day 2 n=12, day 14 n=14; exposed treatment day 2 n=15, day 14 n=13), d) winter 2014, 150 in^3 standard pressure air gun experiment males (control treatment n=11, same individuals sampled at days 0, 2 and 14; exposed treatment n=10, same individuals sampled at days 0, 2 and 14), e) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at days 0-14 post-exposure (control treatment n=11, same individuals sampled at day 0, 2, 14; exposed treatment n=10, same individuals sampled at days 0, 2, 14) and f) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at day 120 post-exposure (control treatment n=11; exposed treatment n=10). For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (a, b, c) or mixed-design ANOVAs (d, e). Significant differences in response to exposure as determined using Welch two-sample t-test (f) are also indicated with a horizontal bar.

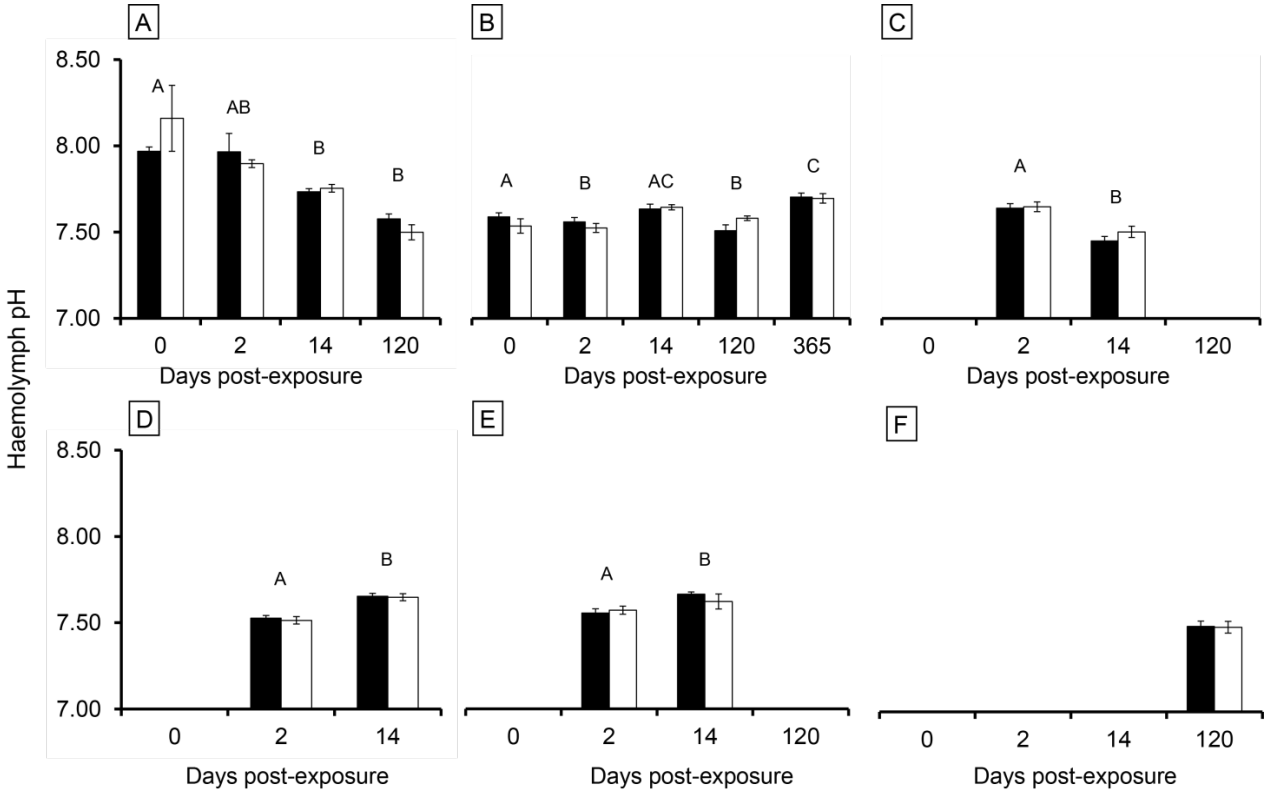


Figure 35. Mean (± SEM) haemolymph pH in lobsters from control (black) and exposed (white) treatments at days 0, 2, 14, 120 and 365 from the a) winter 2013, 45 in^3 standard pressure air gun experiment (control treatment day 0 n=11, day 2 n=10, day 14 n=10, day 120 n=11; exposed treatment day 0 n=10, day 2 n=10, day 14 n=11, day 120 n=10), b) winter 2014, 150 in^3 low pressure air gun experiment (control treatment day 0 n=9, day 2 n=9, day 14 n=8, day 120 n=8 day 365 n=7; exposed treatment day 0 n=8, day 2 n=8, day 14 n=8, day 120 n=7, day 365 n=10) , c) summer 2015, 150 in^3 standard pressure air gun experiment (control treatment day 2 n=12, day 14 n=14; exposed treatment day 2 n=15, day 14 n=13), d) winter 2014, 150 in^3 standard pressure air gun experiment males (control treatment n=11, same individuals sampled at days 0, 2 and 14; exposed treatment n=10, same individuals sampled at days 0, 2 and 14), e) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at days 0-14 post-exposure (control treatment n=11, same individuals sampled at day 0, 2, 14; exposed treatment n=10, same individuals sampled at days 0, 2, 14) and f) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at day 120 post-exposure (control treatment n=11; exposed treatment n=10). For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (a, b, c) or mixed-design ANOVAs (d, e). Significant differences in response to exposure as determined using Welch two-sample t-test (f) are also indicated with a horizontal bar.

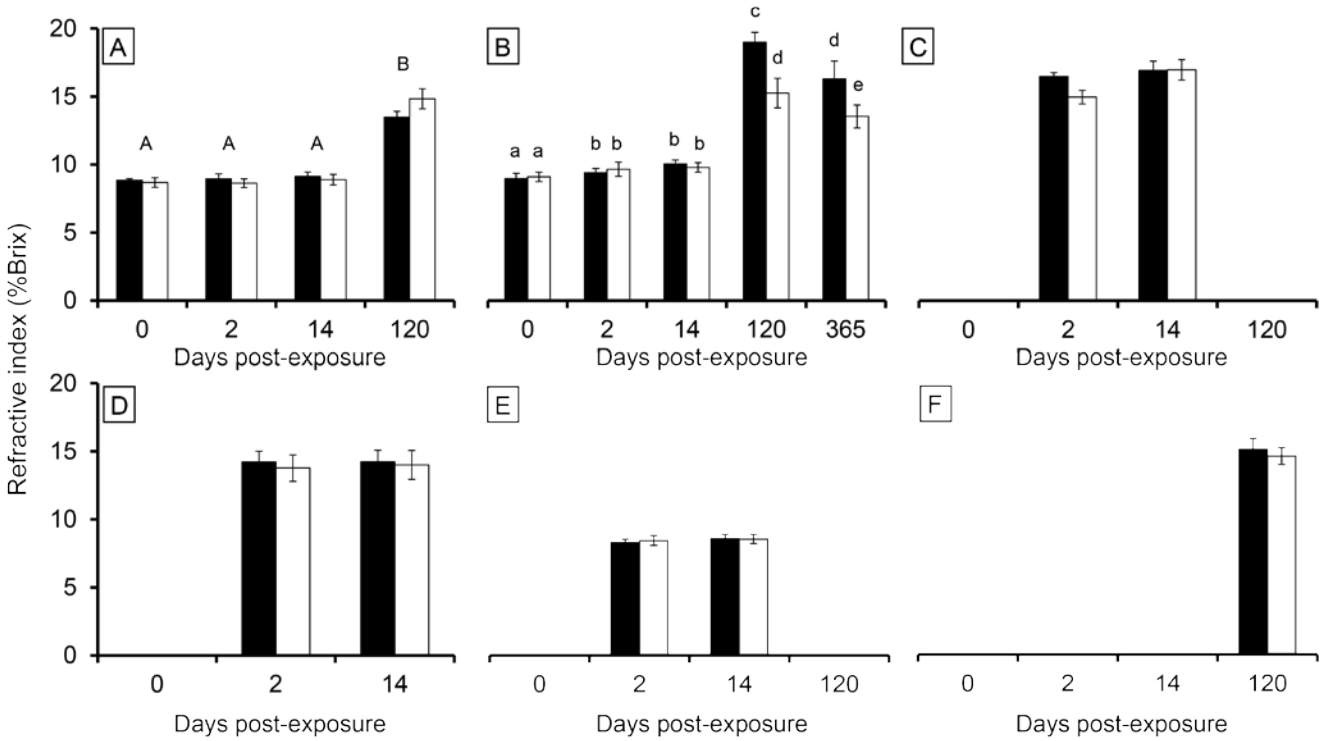


Figure 36. Mean (± SEM) haemolymph refractive index (%Brix) in lobsters from control (black) and exposed (white) treatments at days 0, 2, 14, 120 and 365 from the a) winter 2013, 45 in^3 standard pressure air gun experiment (control treatment day 0 n=11, day 2 n=10, day 14 n=10, day 120 n=11; exposed treatment day 0 n=10, day 2 n=10, day 14 n=11, day 120 n=10), b) winter 2014, 150 in^3 low pressure air gun experiment (control treatment day 0 n=9, day 2 n=9, day 14 n=8, day 120 n=8 day 365 n=7; exposed treatment day 0 n=8, day 2 n=8, day 14 n=8, day 120 n=7, day 365 n=10) , c) summer 2015, 150 in^3 standard pressure air gun experiment (control treatment day 2 n=12, day 14 n=14; exposed treatment day 2 n=15, day 14 n=13), d) winter 2014, 150 in^3 standard pressure air gun experiment males (control treatment n=11, same individuals sampled at days 0, 2 and 14; exposed treatment n=10, same individuals sampled at days 0, 2 and 14), e) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at days 0-14 post-exposure (control treatment n=11, same individuals sampled at day 0, 2, 14; exposed treatment n=10, same individuals sampled at days 0, 2, 14) and f) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at day 120 post-exposure (control treatment n=11; exposed treatment n=10). For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (a, b, c) or mixed-design ANOVAs (d, e). Significant differences in response to exposure as determined using Welch two-sample t-test (f) are also indicated with a horizontal bar.

Table 12. Haemolymph biochemistry parameters for lobsters from winter 2014, 150 in^3 low pressure air gun experiment with female lobsters. Values are reported as mean ± SEM. Significant differences are marked by different letters for exposure or colour for time, as determined using two-way ANOVA or Kruskal-Wallis tests. P values are indicated with asterisks (*P=0.05;P=0.01; ***P<0.001).**

Time (days)	0		2		14		120		Statistics
Treatment	**Control**	**Exposed**	**Control**	**Exposed**	**Control**	**Exposed**	**Control**	**Exposed**	**2-way ANOVA or KW**
Cl (mM)	531.86 ± 15.24	538.13 ± 10.80	527.63 ± 13.92	552.38 ± 19.26	518.88 ± 7.84	530.63 ± 4.11	490.43 ± 12.84	525.00 ± 21.07	NS
K (mM)	6.77 ± 0.16	6.75 ± 0.25	6.94 ± 0.34	7.39 ± 0.35	7.84 ± 0.45	7.31 ± 0.29	7.84 ± 0.66	8.57 ± 1.10	NS
Na (mM)	526.29 ± 12.99	530.63 ± 9.74	525.00 ± 11.32	545.25 ± 16.47	523.88 ± 5.72	532.13 ± 4.64	497.14 ± 7.31	522.00 ± 10.18	NS
Na:K	77.57 ± 1.13	79.25 ± 2.53	76.88 ± 3.11	75.25 ± 3.37	68.50 ± 3.55	73.38 ± 2.58	66.00 ± 4.66	65.43 ± 5.92	df=3,56, F=4.89, Time**
Mg (mM)	9.96 ± 0.35	9.41 ± 0.19	10.31 ± 0.31	10.06 ± 0.14	10.17 ± 0.30	9.76 ± 0.17	10.05 ± 0.28	15.62 ± 5.76	NS
Bicarb (mM)	4.44 ± 0.68	4.64 ± 0.91	6.21 ± 1.15	6.00 ± 1.21	6.75 ± 0.90	6.13 ± 0.40	6.98 ± 2.54	7.73 ± 1.71	NS
Ca (mM)	13.28 ± 0.19	13.31 ± 0.12	13.59 ± 0.19	13.61 ± 0.25	14.40 ± 0.14	14.08 ± 0.15	17.94 ± 1.63	15.33 ± 0.84	df=3,56, F=15.07, Time***
Phos (mM)	0.21 ± 0.03	0.21 ± 0.02	0.41 ± 0.09	0.63 ± 0.21	0.40 ± 0.09	0.23 ± 0.02	1.33 ± 0.51	0.77 ± 0.18	df=3,50, F=10.54, Time***
TP (g l^{-1})	36.00 ± 2.68	35.38 ± 2.17	36.88 ± 2.76	40.50 ± 3.96	40.13 ± 1.73	40.88 ± 2.64	90.33 ± 8.63	72.29 ± 14.08	df=3,51, F=46.82, Time***
Trig (mM)	0.21 ± 0.07	0.15 ± 0.01	0.15 ± 0.02	0.19 ± 0.02	0.31 ± 0.04	0.27 ± 0.02	0.79 ± 0.09	0.88 ± 0.20	KW, Time***
Chol (mM)	0.27 ± 0.02	0.27 ± 0.02	0.26 ± 0.03	0.28 ± 0.02	0.37 ± 0.04	0.36 ± 0.03	0.83 ± 0.15	0.71 ± 0.13	KW, Time***
Gluc (mM)	0.71 ± 0.08	0.60 ± 0.05	0.78 ± 0.13	0.71 ± 0.11	0.56 ± 0.05	0.78 ± 0.05	1.00 ± 0.06	1.16 ± 0.27	df=3,52, F=5.05, Time**
Lactate (mM)	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	0.19 ± 0.05	0.17 ± 0.05	0.89 ± 0.31	0.44 ± 0.11	df=3,33, F=11.16, Time***
Urea (mM)	0.01 ± 0.01	0.06 ± 0.02	0.11 ± 0.02	0.11 ± 0.03	0.11 ± 0.01	0.10 ± 0.00	0.10 ± 0.00	0.11 ± 0.01	df=3,56, F=7.59, Time***
Uric (µM)	28.71 ± 3.05	32.50 ± 2.80	46.00 ± 18.70	84.88 ± 23.29	12.25 ± 2.29	20.00 ± 3.02	45.20 ± 7.23	32.57 ± 7.43	NS
AMY (U l^{-1})	0.43 ± 0.20	0.00 ± 0.00	0.25 ± 0.16	0.25 ± 0.16	0.25 ± 0.16	0.38 ± 0.18	0.83 ± 0.48	0.43 ± 0.20	NS
LIP (U l^{-1})	4.71 ± 0.64	5.25 ± 0.49	5.38 ± 0.42	4.38 ± 0.78	6.38 ± 0.38	5.25 ± 0.31	5.83 ± 0.95	6.57 ± 0.48	NS

ALP (U l^{-1})	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.13	0.13 ± 0.13	0.75 ± 0.16	0.38 ± 0.18	0.00 ± 0.00	0.14 ± 0.14	KW, Time***
AST (U l^{-1})	0.57 ± 0.30	0.63 ± 0.26	0.63 ± 0.26	1.00 ± 0.33	0.25 ± 0.16	0.25 ± 0.16	1.00 ± 0.68	2.29 ± 1.25	NS
ALT (U l^{-1})	0.14 ± 0.14	0.00 ± 0.00	0.38 ± 0.18	0.25 ± 0.16	0.13 ± 0.13	0.00 ± 0.00	0.67 ± 0.67	0.00 ± 0.00	NS
GGT (U l^{-1})	0.86 ± 0.26	0.88 ± 0.23	1.00 ± 0.27	1.63 ± 0.26	1.25 ± 0.16	1.63 ± 0.26	2.00 ± 0.58	1.57 ± 0.53	NS
GDH (U l^{-1})	12.86 ± 2.68	12.88 ± 1.78	13.13 ± 1.90	13.75 ± 2.59	12.50 ± 2.07	11.25 ± 1.11	30.17 ± 6.50	19.29 ± 3.66	df=3,51, F=10.29, Time***
SDH (U l^{-1})	0.00 ± 0.00	0.13 ± 0.13	0.00 ± 0.00	0.13 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.20	0.71 ± 0.29	NS

Table 13. Haemolymph biochemistry parameters for lobsters from the winter 150in^3 standard pressure air gun experiment, with values, reported as mean ± SEM, from males at day 2 and for female lobsters at day 120. Significant differences are marked by different letters for exposure or colour for time, as determined using two-way ANOVA or Kruskal-Wallis tests. P values are indicated with asterisks (*P=0.05;P=0.01; ***P<0.001).**

Time (Days)	**2**				**120**		**Sex × Exposure**	**Day × Exposure**
Sex	Male		Female		Female		Day 2 only	Female only
Treatment	Control	Exposed	Control	Exposed	Control	Exposed	2-way ANOVA or KW	2-way ANOVA or KW
Cl (mM)	489.60 ± 9.52	488.10 ± 7.18	535.80 ± 10.06	472.67 ± 59.92	464.57 ± 18.74	482.40 ± 12.99	NS	NS
K (mM)	7.92 ± 0.45	8.46 ± 0.33	9.36 ± 0.33	7.67 ± 0.99	8.91 ± 0.42	9.00 ± 0.31	NS	NS
Na (mM)	501.90 ± 7.62	498.90 ± 5.88	531.30 ± 8.89	457.00 ± 58.59	474.00 ± 15.86	489.60 ± 11.94	NS	NS
Na:K	64.90 ± 3.21	59.90 ± 2.69	57.40 ± 1.59	55.11 ± 7.40	53.29 ± 1.44	54.80 ± 1.85	NS	NS
Mg (mM)	10.53 ± 0.24	11.21 ± 0.41	9.98 ± 0.27	10.32 ± 0.39	9.12 ± 0.24	9.66 ± 0.27	df=1,34, F=4.61, Sex*	df=1,25, F=4.96, Time*
Bicarb (mM)	8.56 ± 1.81	8.75 ± 1.67	4.28 ± 0.51	4.47 ± 0.61	3.52 ± 0.71	4.94 ± 1.63	NS	NS
Ca (mM)	16.06 ± 0.40	16.01 ± 0.55	13.67 ± 0.19	13.76 ± 0.25	15.32 ± 0.32	15.66 ± 0.27	df=1,34, F=36.01, Sex***	df=1,25, F=46.13, Time***
Phos (mM)	1.06 ± 0.15	0.93 ± 0.16	0.22 ± 0.02	0.28 ± 0.07	0.86 ± 0.29	0.83 ± 0.26	df=1,34, F=47.66, Sex***	df=1,23, F=30.28, Time***
TP (g l^{-1})	73.70 ± 5.84	69.60 ± 7.08	30.44 ± 1.92	29.33 ± 2.67	71.83 ± 6.60	80.80 ± 4.83	df=1,34, F=67.17, Sex***	df=1,25, F=143.44, Time***
Trig (mM)	0.48 ± 0.06	0.51 ± 0.06	0.13 ± 0.02	0.10 ± 0.01	0.96 ± 0.24	0.90 ± 0.13	df=1,34, F=57.95, Sex***	df=1,25, F=100.71, Time***
Chol (mM)	0.54 ± 0.05	0.54 ± 0.06	0.22 ± 0.02	0.21 ± 0.02	0.68 ± 0.12	0.72 ± 0.10	df=1,34, F=70.38, Sex***	df=1,25, F=32.43, Time***
Gluc (mM)	0.56 ± 0.05	0.52 ± 0.04	0.34 ± 0.02	0.37 ± 0.04	1.02 ± 0.10	0.98 ± 0.09	df=1,34, F=21.81, Sex***	df=1,25, F=113.43, Time***
Lactate (mM)	0.13 ± 0.06	0.15 ± 0.05	0.05 ± 0.02	0.09 ± 0.04	1.84 ± 0.47	1.28 ± 0.31	NS	df=1,25, F=70.46, Time***

Urea (mM)	0.08 ± 0.01	0.10 ± 0.02	0.07 ± 0.02	0.08 ± 0.01	0.10 ± 0.00	0.08 ± 0.02	NS	NS
Uric (µM)	23.90 ± 3.10	25.00 ± 2.99	18.33 ± 2.44	26.00 ± 2.74	35.00 ± 7.68	38.40 ± 2.38	NS	df=1,25, F=7.52, Time*
AMY (U l^{-1})	0.30 ± 0.15	0.70 ± 0.15	0.33 ± 0.17	0.56 ± 0.34	0.17 ± 0.17	1.00 ± 0.77	NS	NS
LIP (U l^{-1})	7.60 ± 0.65	6.20 ± 0.44	6.67 ± 0.41	7.00 ± 0.44	9.00 ± 0.63	7.80 ± 0.37	NS	df=1,25, F=10.21, Time***
ALP (U l^{-1})	0.30 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	NS	NS
AST (U l^{-1})	0.50 ± 0.27	0.70 ± 0.21	0.22 ± 0.15	0.78 ± 0.32	6.33 ± 1.96	8.00 ± 1.14	NS	df=1,14, F=22.86, Time***
ALT (U l^{-1})	0.70 ± 0.33	0.60 ± 0.22	0.11 ± 0.11	0.11 ± 0.11	0.33 ± 0.21	1.20 ± 0.49	NS	NS
GGT (U l^{-1})	1.50 ± 0.27	1.40 ± 0.22	1.00 ± 0.00	0.67 ± 0.24	1.83 ± 0.31	1.20 ± 0.49	NS	KW, Time*
GDH (U l^{-1})	11.80 ± 1.38	11.50 ± 1.67	8.67 ± 0.41	10.11 ± 1.10	29.17 ± 3.57	23.80 ± 5.60	NS	KW, Time***
SDH (U l^{-1})	0.20 ± 0.13	0.20 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.17	0.40 ± 0.24	NS	NS

Condition

In the winter 2013 45 in^3 standard pressure experiment, hepatopancreas index (HPI; Figure 37A) was found to be affected significantly by sample time ($F(3,95)=30.895$, $P<0.001$), with significant differences between all four sample times as HPI increased in a stepwise fashion. In the winter 2014 150 in^3 low pressure experiment (Fig. 37B), HPI again responded significantly to sample time ($F(4,70)=12.969$, $P<0.001$), as HPI again increased in a stepwise fashion, although the significant differences were limited to those between day 0 and days 14, 120 and 365; and between day 2 and days 14 and 120. In the summer 2015 150 in^3 standard pressure experiment (Fig. 37C), exposure and sample time were found to have a significant interaction on HPI ($F(1,50)=4.858$, $P=0.03$). Post hoc analysis indicated that this difference was a result of a significant, 5% reduction in HPI in the exposed treatment lobsters between days 2 and 14. In the winter 2014, 150 in^3 standard pressure experiment, HPI was only assessed at a single point, at day 14 for males (Fig. 37D) and females sampled at days 0-14 (Fig. 37E) and at day 120 for females sampled at day 120. No differences were found between treatments in any of these three groups.

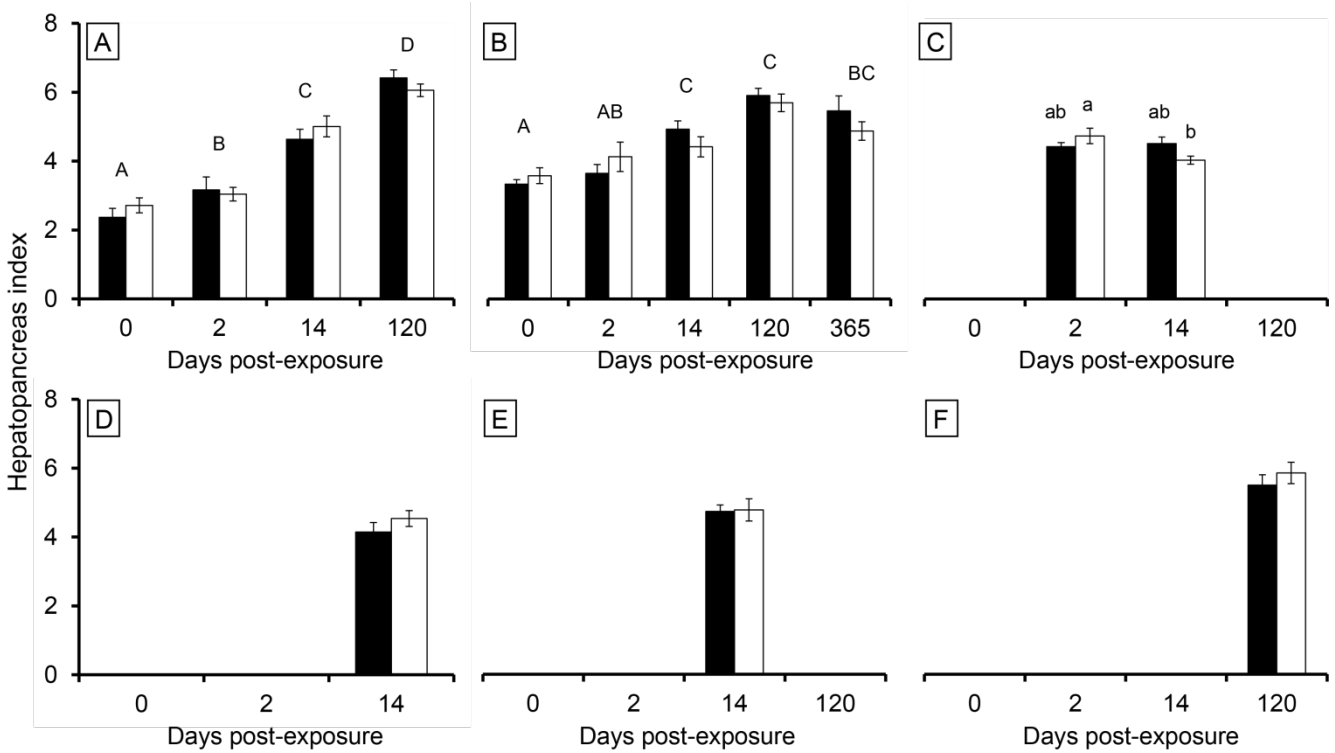


Figure 37. Mean (± SEM) hepatopancreas index ([HP mass · body mass^{-1}] · 100) in lobsters from control (black) and exposed (white) treatments at days 0, 2, 14, 120 and 365 from the a) winter 2013, 45 in^3 standard pressure air gun experiment (control treatment day 0 n=11, day 2 n=10, day 14 n=10, day 120 n=11; exposed treatment day 0 n=10, day 2 n=10, day 14 n=11, day 120 n=10), b) winter 2014, 150 in^3 low pressure air gun experiment (control treatment day 0 n=9, day 2 n=9, day 14 n=8, day 120 n=8 day 365 n=7; exposed treatment day 0 n=8, day 2 n=8, day 14 n=8, day 120 n=7, day 365 n=10) , c) summer 2015, 150 in^3 standard pressure air gun experiment (control treatment day 2 n=12, day 14 n=14; exposed treatment day 2 n=15, day 14 n=13), d) winter 2014, 150 in^3 standard pressure air gun experiment males (control treatment n=11, same individuals sampled at days 0, 2 and 14; exposed treatment n=10, same individuals sampled at days 0, 2 and 14), e) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at days 0-14 post-exposure (control treatment n=11, same individuals sampled at day 0, 2, 14; exposed treatment n=10, same individuals sampled at days 0, 2, 14) and f) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at day 120 post-exposure (control treatment n=11; exposed treatment n=10). For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (a, b, c) or mixed-design ANOVAs (d, e). Significant differences in response to exposure as determined using Welch two-sample t-test (f) are also indicated with a horizontal bar.

Embryonic development, quantity and quality

Relative egg bundle size

Bundle index (Fig. 38), which compared the mass of the egg bundle to body mass, was found to respond significantly to sample time in the 2013 45 in^3 standard pressure experiment ($F(2,56)=21.381$, $P<0.001$), with lobsters at day 14 showing a significantly greater BI than day 0 and day 2 lobsters (Fig. 38A).

In the winter 2014, 150 in^3 low pressure experiment (Fig. 38B), bundle index was again significantly affected by sample time ($F(3,47)=5.706$, $P=0.002$), as day 2 lobsters showed a significantly lower BI compared to day 0 and day 365 lobsters.

In the winter 2014, 150 in^3 standard pressure experiment (Fig. 38C), bundle index was not significantly different at day 14 ($t(18.31)=0.93$, $P=0.37$).

Hatching and fecundity

There were no mortalities of the adult berried female lobsters in either control or exposed treatments for any of the three experiments. Similarly, all females had successful hatches with no incidence of loss or removal of the egg bundle. Lobsters in both treatments over all three experiments hatched over the course of a 5-6 day period, with a peak in the number of larvae hatched around days 3-4.

Comparison of the number of larvae hatched (Fig. 39A) between all treatments using ANCOVA with carapace length (CL) as the covariate showed that the mean number of hatched larvae differed significantly ($F(5,46)=4.437$, $P <0.003$) with CL significantly related to fecundity ($F(1,46)=14.123$, $P<0.001$). However, differences in fecundity were limited to comparisons between experiments, with no differences between control and exposed treatments within an experiment.

Larval morphology

Observation of larval morphology revealed no abnormalities in any of the hatches. Comparisons of larval body length (Fig. 39B) showed significant differences ($F(5,47)=22.52$, $P<0.001$) between treatments. Tukey HSD post hoc analysis showed a significant difference between control and exposed larvae in the winter 2013, 45 in^3 standard pressure experiment, as exposed larvae were approximately 1.5% longer than control. When compared between experiments, control larvae from the winter 2013, 45 in^3 standard pressure experiment were significantly longer than both control and exposed treatments from the winter 2014, 150 in^3 low pressure experiment, by 1.4% and 1.3% respectively, and exposed larvae from the winter 2013, 45 in^3 standard pressure experiment were significantly longer than larvae from any of the other treatments by about 2.8% compared to the winter 2014 150 in^3 low pressure treatments and about 2% in the winter 2014 150 in^3 standard pressure treatments. There were no differences in larval length between control and exposed treatments in the winter 2014, 150 in^3 low and standard pressure experiments.

Larval width (Fig. 39C) also showed a significant interaction between exposure and sample time ($F(5,47)=15.192$, $P<0.001$). In this case, no differences were found between control and exposed treatments within any of the three experiments. Comparisons between the three experiments showed that larvae from the control treatment of the winter 2013, 45 in^3 standard pressure experiment had a significantly greater width than larvae from both treatments of the winter 2014, 150 in^3 low pressure experiment by approximately 1.9% and from both treatments of the winter 2014, 150 in^3 standard pressure experiment by approximately 1.4%. Larvae from the exposed treatment of the winter 2013, 45 in^3 standard pressure experiment were significantly wider than larvae from both treatments of the winter 2014, 150 in^3 low pressure experiment by approximately 2.4% and from both treatments of the winter 2014, 150 in^3 standard pressure experiment by approximately 1.9%.

Length-to-weight and width-to-weight ratios were compared between treatments for all three experiments; however, as there were no differences apparent, these data are not shown.

Larval dry mass and energy

Contrary to the results of larval length and width comparisons, no significant differences were found within or between the dry masses (Fig. 39D) of any of the treatments ($F(5,49)=1.751$, $P=0.15$). Similarly, larval energy content (Fig. 39E) did not differ between treatments in any of the exposure levels when compared using ANOVA ($F(5,44)=1.493$, $P=0.212$).

Larval competency

No difference was found in larval competency, as measured through survival time in an elevated temperature and reduced salinity activity test (Smith *et al.* 2003b), between control and exposed larval treatments from the winter 2013, 45 in^3 standard pressure experiment (Fig. 40A). Both treatments had a median survival time of 24 min and the hazard ratio, which compares the slope of the survival curves and thus the rate of death, was 1.129 with a 95% confidence interval (95%CI) of 0.9742, 1.308 for control larvae and 0.8860 with a 95%CI of 0.7647, 1.026 for exposed larvae. These hazard ratio results reflect the proportion of deaths occurring at any given point in one treatment relative to the other treatment—i.e. at any given time, the probability of a control larvae death was 1.129 times that of an exposed larvae. Again, there was no difference in the activity test results between control and exposed larvae from the winter 2014, 150 in^3 low pressure experiment (Fig. 40B). Both treatments had a 21 min mean survival time and a hazard ratio of 1.002 with a 95%CI of 0.8846, 1.139 for control larvae and 0.9978 with a 95%CI of 0.8777, 1.131 for exposed. Similarly, no difference was found in activity results for winter 2014, 150 in^3 standard pressure experiment larvae (Fig. 40C), with median survival of 18 min for both control and exposed larvae and hazard ratio of 0.9397 95%CI 0.7795, 1.017 for control and 1.064 95%CI 0.9829, 1.283 for exposed treatments.

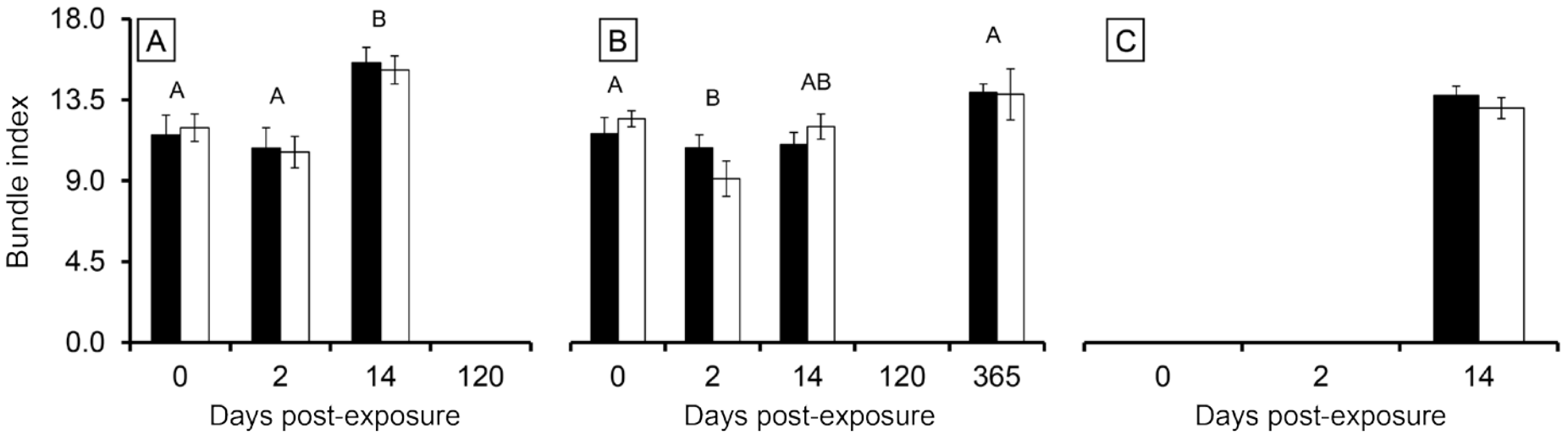


Figure 38. Mean (± SEM) bundle index ([egg bundle mass · body mass^{-1}] · 100) in lobsters from control (black) and exposed (white) treatments at days 0, 2, 14, 120 and 365 from the a) winter 2013, 45 in^3 standard pressure air gun experiment (control treatment day 0 n=11, day 2 n=10, day 14 n=10, day 120 n=11; exposed treatment day 0 n=10, day 2 n=10, day 14 n=11, day 120 n=10), b) winter 2014, 150 in^3 low pressure air gun experiment (control treatment day 0 n=9, day 2 n=9, day 14 n=8, day 120 n=8 day 365 n=7; exposed treatment day 0 n=8, day 2 n=8, day 14 n=8, day 120 n=7, day 365 n=10) , c) winter 2014, 150 in^3 standard pressure air gun experiment berried females sampled at days 0-14 post-exposure (control treatment n=11, same individuals sampled at day 0, 2, 14; exposed treatment n=10, same individuals sampled at days 0, 2, 14). For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (a, b). Significant differences in response to exposure as determined using Welch two-sample t-test (c) are also indicated with a horizontal bar.

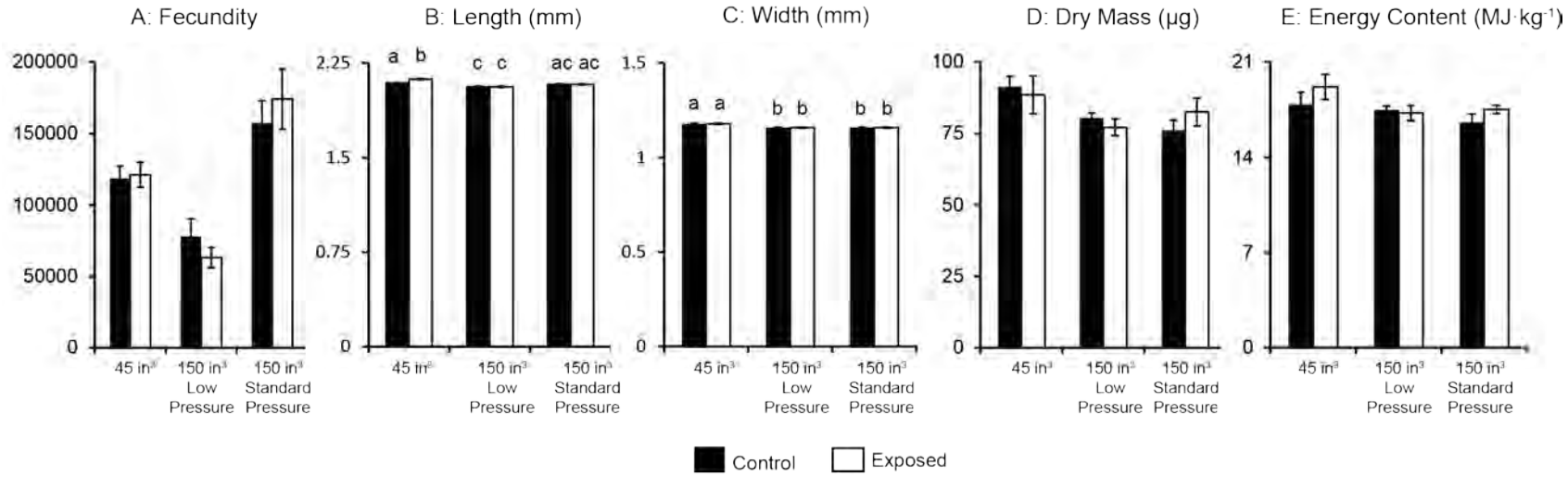


Figure 39. Comparisons of measurements of newly hatched lobster larvae from the winter 2013, 45 in^3 standard pressure air gun experiment, the winter 2014, 150 in^3 low pressure air gun experiment and the winter 2014, 150 in^3 standard pressure air gun experiment. Mean (A) female fecundity (winter 2013 45 in^3 standard pressure control n=10, exposed n=10; winter 2014 150 in^3 low pressure control n=7, exposed n=10; winter 2014 150 in^3 standard pressure control n=11, exposed n=10), (B) larval length (winter 2013 45 in^3 standard pressure control n=200, exposed n=200; winter 2014 150 in^3 low pressure control n=140, exposed n=200; winter 2014 150 in^3 standard pressure control n=220, exposed n=200), (C) larval width (winter 2013 45 in^3 standard pressure control n=200, exposed n=200; winter 2014 150 in^3 low pressure control n=140, exposed n=200; winter 2014 150 in^3 standard pressure control n=220, exposed n=200), (D) larval dry mass (winter 2013 45 in^3 standard pressure control n=10, exposed n=10; winter 2014 150 in^3 low pressure control n=7, exposed n=10; winter 2014 150 in^3 standard pressure control n=11, exposed n=10) and (E) larval energy content (winter 2013 45 in^3 standard pressure control n=10, exposed n=10; winter 2014 150 in^3 low pressure control n=7, exposed n=10; winter 2014 150 in^3 standard pressure control n=11, exposed n=10) with error bars indicated SEM. Larval length was significantly different between control and exposed treatments for the winter 2013, 45 in^3 standard pressure experiment as determined using a nested ANOVA and is indicated with an asterisk.

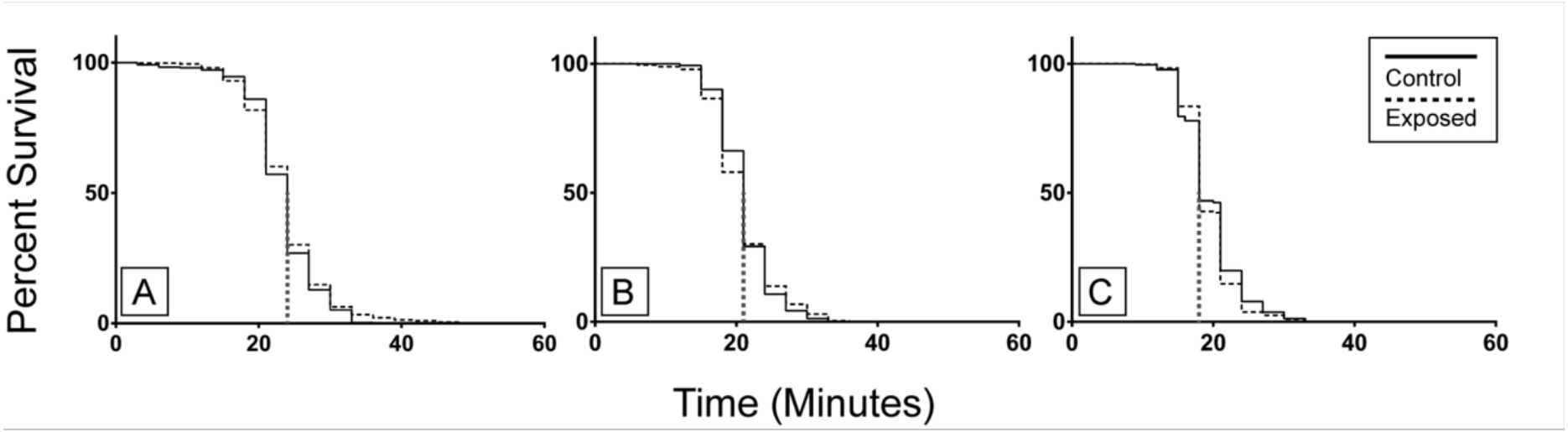


Figure 40. Percent survival during the activity test of newly hatched lobster larvae from the winter 2013, 45 in^3 standard pressure air gun experiment, the winter 2014, 150 in^3 low pressure air gun experiment and the winter 2014, 150 in^3 standard pressure air gun experiment. Kaplan-Meier survival analysis of larval activity test indicates the estimated percentage of larvae surviving over the test time period, in minutes, for control and exposed larvae. Median survival time for each experiment, which in all cases is the same for control and exposed treatments within the experiment, is indicated by a vertical grey dashed line. For the winter 2013, 45 in^3 standard pressure experiment, each curve represents 600 larvae. For the 150 in^3 low pressure experiment the control curve represents 400 larvae and the exposed curve represents 600 larvae. For the 2014, 150 in^3 standard pressure experiment, the control curve represents 480 larvae and the exposed curve represents 400 larvae. For all three experiments, the lack of any difference in both the median survival time and the slope of the curves representing the two treatments indicated that there was no difference in level of larval competency.

Scallop Results

Mortality

In the 2013 experiment, of the 53 scallops in the 0-pass treatment, 1 mortality was recorded at day 0, 1 at day 14 post-exposure and 0 at day 120 post-exposure. In the 1-pass treatment, which was also comprised of 53 scallops, there were no mortalities recorded at day 0, 2 at day 14 and 3 at day 120. In the 2-pass treatment, there were 1, 1 and 4 mortalities at days 0, 14 and 120, respectively, out of a total of 53 scallops. In the 4-pass treatment, which had 54 scallops, there were 0 mortalities at day 0, 3 at day 14 and 5 at day 120. At the end of this experiment, the cumulative mortalities were 3.8%, 9.4%, 11.3% and 14.8% for 0-pass control, 1-pass, 2-pass and 4-pass treatments respectively (Fig. 41).

In 2014, the 0-pass treatment had 55 scallops, of which 0 were found dead at day 0, 1 at day 14 and 1 and day 120. In the 1-pass treatment, of 53 scallops, 0 mortalities were recorded at day 0, 2 mortalities were recorded at day 14 and 4 mortalities were recorded at day 120. The 2-pass treatment had 56 scallops, and at the 0, 14 and 120 day post-exposure sample points, 2, 2 and 5 mortalities were recorded. In the 4-pass treatment, there were 0 mortalities at day 0, 3 at day 14 and 7 at day 120, from a total of 57 scallops. The cumulative mortality at the conclusion of this experiment was 3.6%, 11.3%, 16.1% and 17.5% for 0-pass, 1-pass, 2-pass and 4-pass treatments (Fig. 41).

For the 2015 experiment, mortality was only recorded at day 14 as both 0- and 4-pass treatments suffered complete mortality prior to the day 120 sample point. Each treatment was comprised of 20 scallops, with 1 mortality recorded in the 0-pass treatment for a 5% mortality rate and 4 mortalities recorded in the 4-pass treatment for a 20% mortality rate.

A binomial regression, restricted to the 2013 and 2014 experiments due to the mass mortality of both 0-pass and 4-pass treatments in the 2015 experiment, found no significant difference in mortality rates between 2013 and 2014 ($P=0.48$), a significant increase in the mortality probability through time ($P<0.001$) and a significant difference in mortality rates between exposure levels ($P=0.009$). Compared with unexposed scallops, the daily mortality odds were found to be 0.1%, 1.2% and 1.3% higher in scallops exposed to 1, 2 and 4 passes respectively.

Haemocyte counts

In the 2013 experiment, analysis of total haemocyte counts (THC, Figure 42) showed that the interaction between exposure and sample time was significant ($F(6,182)=3.54$, $P=0.002$), with post hoc analysis showing significant differences between the mean THC of 0-pass scallops at day 0 and that of 2-pass and 4-pass scallops, as the 0-pass treatment showed THC levels 73% and 75% greater, respectively. At day 14, there were no significant differences between treatments. At day 120, 0-pass scallops had a 60% increase over day 0 THC levels and a 119% increase over day 14 levels, both of which were significant differences. Furthermore, the 0-pass controls demonstrated a significantly greater THC compared to the 1-pass, 2-pass and 4-pass scallops, with 58%, 59% and 90% higher THC levels, respectively. Between the exposed treatments, mean THC was similar at day 120, though 1-pass showed a significant 64% increase from day 14 levels, and 2-pass scallops

showed a significant increase of *ca.* 75% from both day 0 and day 14 levels. Scallops exposed to 4-passes did not show any difference in THC levels across the 3 sample points.

In 2014, the interaction between exposure level and sample time was again significant ($F(6,174)=17.69$, $P<0.001$). All four treatments showed similar THC levels at day 0. At day 14, mean THC in 4-pass scallops was 41% greater than that of 0-pass scallops, a difference that was significant. At day 120, 0-pass scallops showed a slight, but non-significant increase in mean THC, whereas 1-pass, 2-pass and 4-pass scallops had reductions of 50%, 57% and 61%, respectively, relative to day 0 and reductions of 64%, 66% and 70%, respectively, relative to day 14. These reductions also led to a significant difference between 0-pass scallops and all three exposed treatments at day 120, as 0-pass scallops had nearly 3 fold more circulating haemocytes than each of the exposed treatments.

In the 2015 experiment, THC was only recorded at day 14, with results similar to day 14 of the 2014 experiment, with a mean THC in 4-pass scallops 21% greater than that of 0-pass scallops, a difference that was significant when compared using a Welch Two Sample t-test ($t(33.3)=2.44$, $P=0.03$).

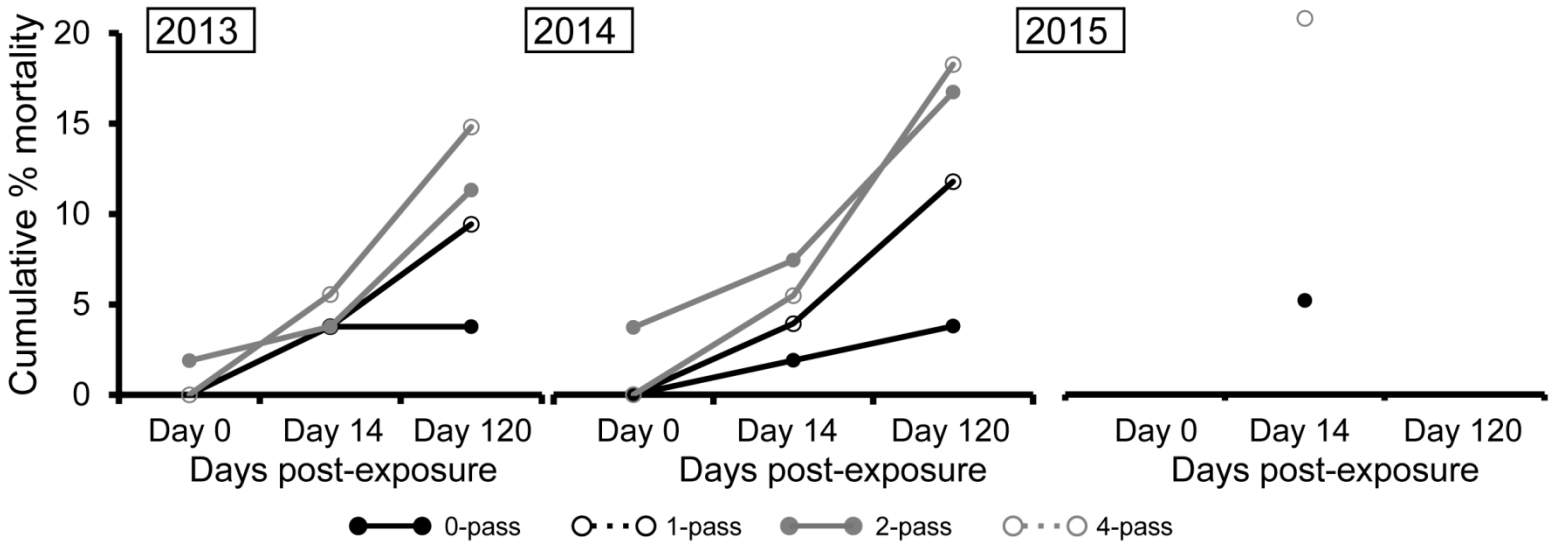


Figure 41. Cumulative scallop mortality percentage for each of the treatment levels at each sample point in the winter 2013 45 in^3 air gun experiment, winter 2014 150 in^3 air gun experiment and summer 2015 150 in^3 air gun experiment. Sample sizes from each treatment in the 2013 experiment were: 0-pass=53, 1-pass=53, 2-pass=53, 4-pass=54; in the 2014 experiment, were: 0-pass=55, 1-pass=53, 2-pass=56, 4-pass=57; and in the 2015 experiment were: 0-pass=20, 4-pass=20.

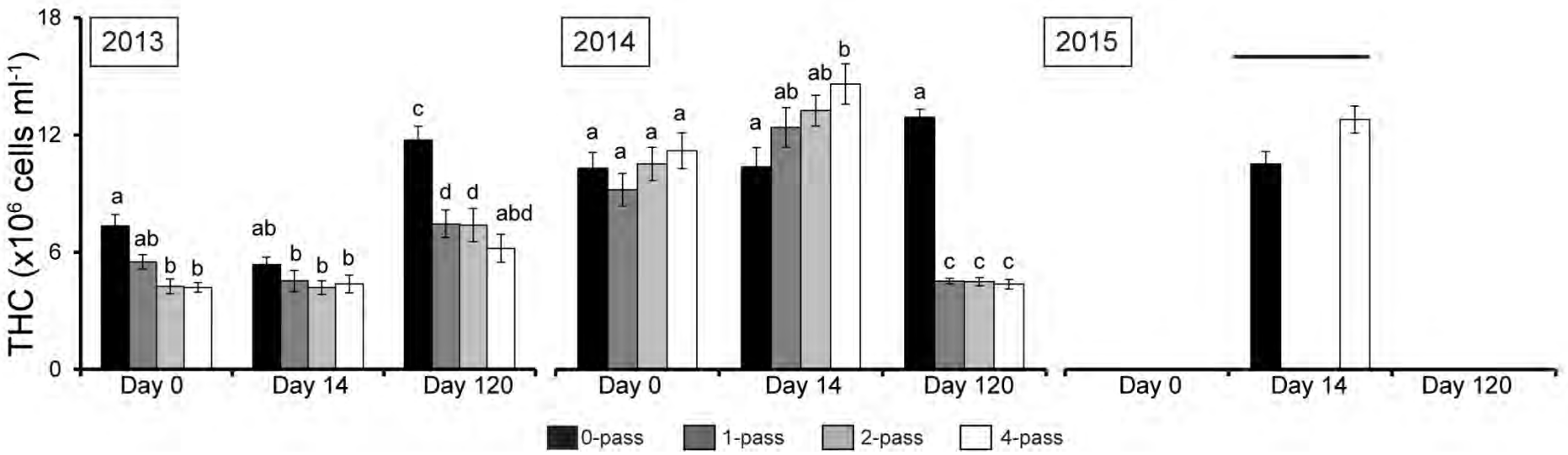


Figure 42. Mean (± SEM) total haemocyte counts (THC) in scallops from the winter 2013 45 in^3 air gun experiment (at day 0, 0-pass n=16, 1-pass n=19, 2-pass n=17, 4-pass n=20; at day 14, 0-pass n=16, 1-pass n=17, 2-pass n=20, 4-pass n=19; at day 120, 0-pass n=17, 1-pass n=15, 2-pass n=16, 4-pass n=14), winter 2014 150 in^3 air gun experiment (at day 0, 0-pass n=20, 1-pass n=19, 2-pass n=17, 4-pass n=19; at day 14, 0-pass n=17, 1-pass n=16, 2-pass n=15, 4-pass n=16; at day 120, 0-pass n=16, 1-pass n=12, 2-pass n=15, 4-pass n=12) and summer 2015 150 in^3 air gun experiment (at day 14, 0-pass n=19, 4-pass n=17). For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (2013, 2014). Significant differences in response to exposure level as determined using Welch two-sample t-test (2015) are also indicated with a horizontal bar.

Haemolymph biochemistry

Haemolymph pH (Fig. 43, top) values for the 2013 experiment showed a decreasing trend across the three sample points, from high pH values at day 0 (> 8.00 for all treatments), to moderately high values at day 14 (between 7.85 and 7.91 for all treatments), to moderate levels by day 120 (between 7.46 and 7.63 for all treatments). Comparison showed a significant interaction for air gun passes and sample time ($F(6,189)=2.307$, $P=0.036$). Pairwise comparison showed that at day 0, 1-pass scallops had a significantly higher pH than the other treatments, which was the only difference between treatments at the three sample points. In addition, all treatments showed a significant reduction in haemolymph pH at day 14 relative to day 0 and at day 120 relative to both day 0 and day 14.

For the 2014 experiment, there was a significant interaction between air gun passes and sample time ($F(6,182)=4.544$, $P<0.001$). Post hoc analysis showed a trend of significant differences within treatments between sample times with a relatively moderate pH on day 0, with means from all four treatments between 7.41 and 7.50; a more acidic pH on day 14, with means between 7.29 and 7.47; and a more alkaline pH on day 120, with means of 7.61-7.68. Additionally, at day 0, the mean pH of 7.41 ± 0.02 in 0-pass scallops was significantly lower than that of 1-pass, 2-pass and 4-pass scallops. At day 14, the mean pH 4-pass scallops, was significantly higher than that of 0-pass, 1-pass and 2-pass scallops. No differences were found between treatments at day 120.

At the lone sample point from the 2015 experiment, day 14, haemolymph pH was significantly different ($t(29.65)=-3.8253$, $P<0.001$) between 0-pass scallops, with a mean pH of 7.43 ± 0.03, and 4-pass scallops, with a mean of 7.61 ± 0.04.

Haemolymph refractive index (Fig. 43, bottom) for the 2013 experiment showed a slight increasing trend across the three sample points within each of the four treatments, with mean values at day 0 between 4.1 and 4.3, between 4.2 and 4.4 at day 14 and between 4.5 and 4.6 at day 120. There was no significant differences between treatments ($F(6,189)=0.54$, $P=0.78$). As a point of comparison, the seawater from the tank scallops were held in had a mean refractive index of 4.3.

For the 2014 experiment, haemolymph refractive index again showed a slight increase with time over the course of the experiment, with means at day 0 approximately 4.4 for all treatments, between 4.5 and 4.7 at day 14 and between 4.6 and 4.8 at day 120. Comparison showed a significant interaction between exposure and sample time ($F(6,182)=9.831$, $P<0.001$). Post hoc analysis revealed the increase in refractive index between days 0 and 14 was significant in all treatments, between days 14 and 120 for 0-pass scallops and between days 0 and 120 for all treatments. Comparing between treatments showed that at day 14, 0- and 1-pass scallops had a significantly lower refractive index than scallops exposed to 2-passes and 4-passes. At day 120, 0-pass scallops had a higher refractive index than either 1-passor 4-pass scallops. Again, seawater from the holding tank had a refractive index of 4.3, with scallops from all treatments across the three sample points showing a somewhat (2-10%) higher refractive index in comparison.

Haemolymph refractive index in 0- and 4-pass scallops did not significantly differ in the 2015 experiment ($t(33.27)=1.13$, $P=0.122$) and was again slightly greater than that of the 4.3 observed for seawater.

Assays of humoral electrolyte and mineral ion levels, which were conducted on samples collected in the winter 2014, 150 in^3 experiment, showed a range of responses to air gun exposure (see Table 14 for mean values and statistics for all ions). Haemolymph sodium (Na) concentration showed a significant response to both exposure ($F(3,179)=8.220$, $P<0.001$) and sample time ($F(2,179)=4.407$, $P=0.014$), but not the interaction of the terms ($F(6,179)=1.930$, $P=0.078$). In terms of exposure, 0-pass scallops had significantly lower Na concentrations than all 3 exposed treatments, with 1-pass, 2-pass and 4-pass scallops showing Na levels 1.1%, 2.5% and 4.6% higher than 0-pass control scallops, respectively. Sample time showed significant differences between day 0 samples and the subsequent 2 sample days, with a 1.0% increase at day 14 and a 3.6% increase at day 120. Exposure level was found to significant affect potassium (K) concentrations ($F(3,179)=8.303$, $P<0.001$), whereas sample time was not ($F(2,179)=1.036$, $P=0.35$) and there was no interaction between the factors ($F(6,179)=0.838$, $P=0.54$). Post hoc analysis showed that the 4-pass scallops had significantly increased K levels compared to the other 3 treatments, exceeding levels in other treatments by 6.5%. Although there were differences in Na and K, there was no significant difference in Na:K ratio, either in response to exposure ($F(3,179)=2.373$, $P=0.072$) or sample time ($F(2,179)=0.126$, $P=0.88$). Chloride ions (Cl) differed significantly as a result of exposure ($F(3,179)=4.1$, $P=0.007$), with 4-pass scallops showing levels of Cl 3.6%, 2.2% and 1.2% higher than those of 0-pass, 1-pass and 2-pass scallops. Magnesium concentration showed a significant interaction between exposure and sample time ($F(6,179)=3.668$, $P=0.0018$). At day 0, 1-pass scallops had a Mg concentration 2.6% greater than that of 0-pass control scallops. By day 14, the Mg concentration in 1-pass scallops decreased by 5.5% before rising by 1.8% by day 120, with both changes showing statistical significance. At day 120, 4-pass scallops also showed a significantly higher Mg concentration than 0-pass scallops at day 0, by 2% and by 1-pass scallops at day 14 by 5% and at day 120 by 3%. Air gun exposure and sample time showed a significant interaction on bicarbonate levels ($F(6,174)=2.274$, $P=0.039$). Between treatments, 1-pass scallops showed bicarbonate levels significantly greater than 2-pass and 4-pass scallops at day 0, with levels 8 and 18% greater, respectively. At day 14, 4-pass scallops had bicarbonate levels 20% lower than that of 0-pass controls. Within each treatment, neither 0-pass nor 1-pass scallops showed any changes in bicarbonate levels over time. Conversely, 2-pass scallops showed a 15% increase between day 0 and day 14 and 4-pass scallops showed a 19% increase between days 0 and 120. Calcium levels showed a significant response to the interaction of exposure and sample time ($F(6,180)=2.891$, $P=0.010$). Post hoc analysis indicated that Ca level did not change in 0-pass, 1-pass or 2-pass scallops over the sample times, whereas 4-pass scallops showed a significant 6.6% increase at day 120 over day 14 levels, which led to Ca concentration about 7% greater than 0-, 1- and 2-pass scallops. Phosphorus levels differed significantly as a result of exposure ($F(3,179)=4.791$, $P=0.003$), though in this case, 2-pass scallops had P levels significantly lower than 0- (18% lower) and 1-pass (12% lower) and 4-pass (30% lower) scallops.

Organic molecules (see Table 15 for all mean values and statistics) showed a more limited response, with significant differences only in total protein and glucose levels. For TP, exposure and sample time displayed a significant interaction ($F(6,178)=2.579$, $P=0.020$), though no significant differences were found amongst relevant treatments and sample times following post hoc analysis. For glucose, exposure had a significant effect ($F(3,180)=5.37$, $P=0.01$), with 0-pass scallops showing glucose levels 13% higher than 1-pass scallops and 41% higher than 4-pass scallops.

No significant differences were observed in any of the enzymes assayed (see Table 16 for all means and statistics), and in most cases, enzyme activity levels were very low with a great deal of variation between individuals. This may indicate that these values are not relevant for scallop haemolymph, however, given the paucity of published haemolymph biochemistry data for scallops, they are reported here nonetheless.

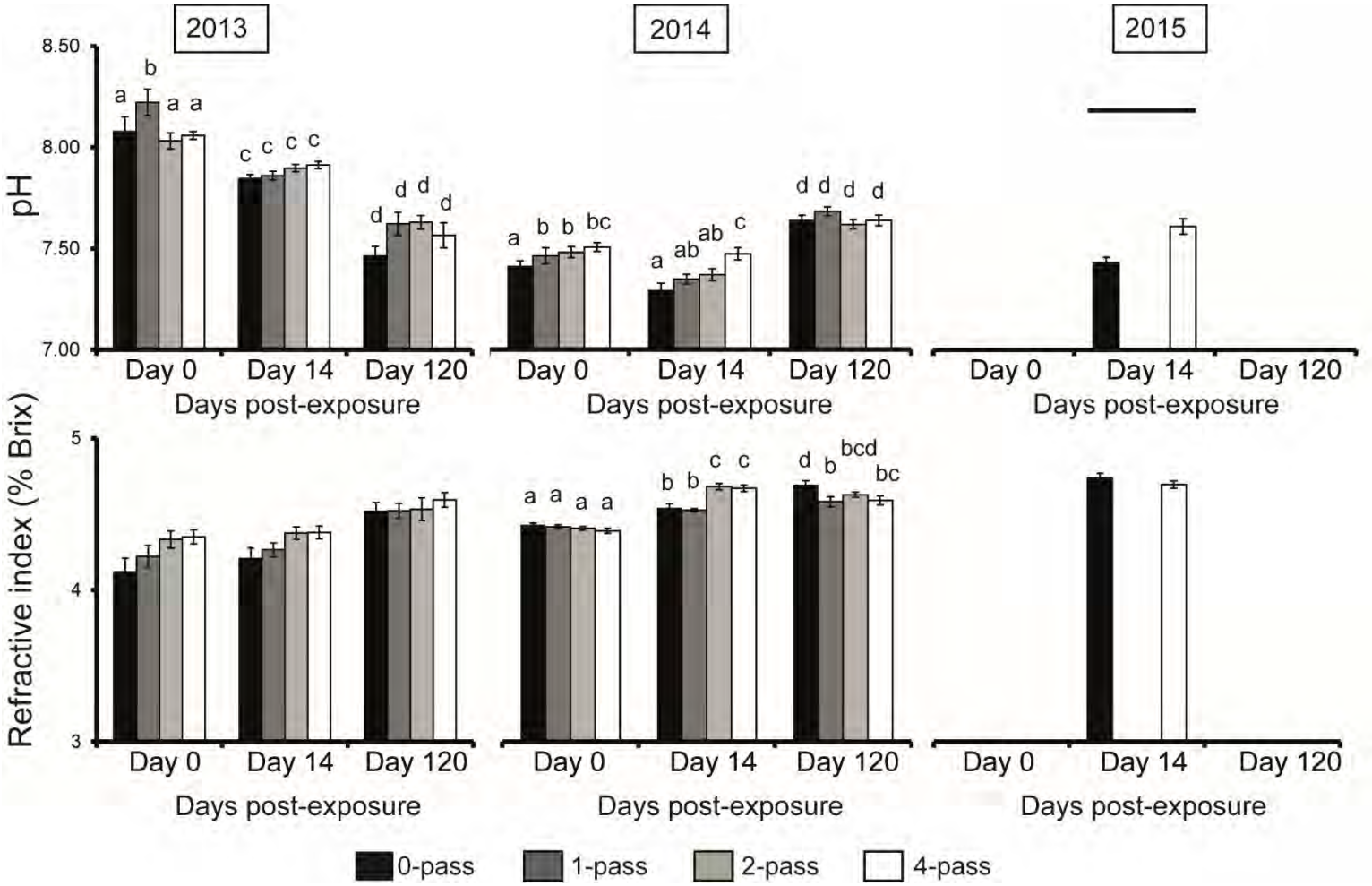


Figure 43. Mean (± SEM)haemolymph pH levels (top) and refractive index (bottom) in scallops from the winter 2013 45 in^3 air gun experiment (at day 0, 0-pass n=16, 1-pass n=19, 2-pass n=17, 4-pass n=20; at day 14, 0-pass n=16, 1-pass n=17, 2-pass n=20, 4-pass n=19; at day 120, 0-pass n=17, 1-pass n=15, 2-pass n=16, 4-pass n=14), winter 2014 150 in^3 air gun experiment (at day 0, 0-pass n=20, 1-pass n=19, 2-pass n=17, 4-pass n=19; at day 14, 0-pass n=17, 1-pass n=16, 2-pass n=15, 4-pass n=16; at day 120, 0-pass n=16, 1-pass n=12, 2-pass n=15, 4-pass n=12) and summer 2015 150 in^3 air gun experiment (at day 14, 0-pass n=19, 4-pass n=17). For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (2013, 2014). Significant differences in response to exposure level as determined using Welch two-sample t-test (2015) are also indicated with a horizontal bar.

Table 14. Haemolymph electrolytes and minerals in scallops from the winter 2014, 150 in^3 air gun experiment, based on exposure level (0-pass, 1-pass, 2-pass, 4-pass) and sample time (0, 14, 120 days post-exposure). All values are expressed as means ± SEM with sample sizes indicated in parentheses. Information regarding the statistical test used (two way ANOVA or randomised permutation test ANOVA), along with identification of any transformation applied to data (BC=Box-Cox transformation with value for lambda indicated, sqrt=square root transformation) is provided. Significant differences in response to exposure level are marked by different colours (dark grey=a, light grey=ab, white=b, black=c), in response to sample time by upper case letters and in response to the interaction between exposure and sample time by lower case letters.

Exposure	0-pass			1-pass			2-pass			4-pass			Test	Significance		
Time (days)	0	14	120	0	14	120	0	14	120	0	14	120		Exposure	Time	Interaction
Na (mmol l^{-1})	468±2 A (20)	471 ± 4 B (17)	476 ± 8 B (15)	474 ± 4 A (19)	466 ± 6 B (15)	484 ± 7 B (10)	476 ± 4 A (18)	480 ± 6 B (16)	488 ± 6 B (15)	484 ± 7 A (18)	496 ± 5 B (15)	504 ± 9 B (13)	rANOVA	df=3,179 F=8.220 P<0.001	df=2,179 F=4.407 P=0.013	NS
K (mmol l^{-1})	11.9 ± 0.2 (20)	11.8 ± 0.3 (17)	11.8 ± 0.2 (15)	11.8 ± 0.2 (19)	11.8 ± 0.3 (15)	12.2 ± 0.4 (10)	11.7 ± 0.2 (18)	11.7 ± 0.3 (16)	12.3 ± 0.2 (15)	12.6 ± 0.3 (18)	12.9 ± 0.1 (15)	12.5 ± 0.1 (13)	Two-way ANOVA	df=3,179 F=8.303 P<0.001	NS	NS
Na:K	39.5 ± 0.7 (20)	40.2 ± 0.9 (17)	40.4 ± 0.7 (15)	40.4 ± 0.5 (19)	39.9 ± 0.9 (15)	39.9 ± 1.2 (10)	40.8 ± 0.6 (18)	41.2 ± 0.7 (16)	39.9 ± 0.5 (15)	38.8 ± 0.7 (18)	38.6 ± 0.4 (15)	40.3 ± 0.7 (13)	Two-way ANOVA	NS	NS	NS
Cl (mmol l^{-1})	543.30 ± 2.75 (15)	544.41 ± 4.94 (15)	553.00 ± 12.59 (15)	551.84 ± 7.58 (15)	543.00 ± 9.16 (15)	567.60 ± 9.38 (15)	553.67 ± 5.92 (15)	559.50 ± 8.31 (15)	566.20 ± 8.66 (15)	540.47 ± 32.25 (15)	560.53 ± 38.50 (15)	587.31 ± 11.89 (15)	Two-way ANOVA BC λ=0.55	df=3,179 F=4.10 P=0.007	NS	NS
Mg (mmol l^{-1})	54.12 ± 0.64 c (20)	54.95 ± 0.90 c (17)	56.21 ± 0.88 c (15)	55.53 ± 0.77 bc (19)	52.50 ± 0.95 a (15)	53.43 ± 0.91 a (10)	52.72 ± 0.50 a (17)	53.54 ± 0.48 a (16)	53.17 ± 0.24 a (15)	53.20 ± 0.32 a (19)	53.73 ± 0.46 a (15)	55.17 ± 0.77 ab (13)	rANOVA			df=6,179 F=3.67 P=0.002
Bicarb (mmol l^{-1})	2.1 ± 0.1 bc (18)	2.2 ± 0.1 c (17)	2.1 ± 0.1 bc (14)	2.0 ± 0.1 b (18)	2.0 ± 0.1 b (15)	2.0 ± 0.1 b (10)	1.8 ± 0.1 ab (17)	2.1 ± 0.1 bc (16)	1.9 ± 0.1 ab (15)	1.6 ± 0.1 a (18)	1.8 ± 0.1 ab (15)	2.0 ± 0.1 b (13)	Two-way ANOVA BC λ=1.35			df=6,174 F=2.274 P=0.04
Ca (mmol l^{-1})	10.33 ± 0.11 a (20)	10.44 ± 0.05 a (17)	10.55 ± 0.09 b (15)	10.47 ± 0.08 a (19)	10.48 ± 0.10 a (15)	10.53 ± 0.14 b (10)	10.49 ± 0.07 a (18)	10.55 ± 0.12 a (16)	10.52 ± 0.04 b (15)	10.52 ± 0.09 a (19)	10.57 ± 0.03 a (15)	11.28 ± 0.24 b (13)	rANOVA			df=6,180 F=2.891 P=0.01
P (mmol l^{-1})	0.42 ± 0.03 (20)	0.38 ± 0.03 (17)	0.38 ± 0.04 (14)	0.39 ± 0.02 (19)	0.41 ± 0.03 (15)	0.43 ± 0.05 (10)	0.35 ± 0.03 (18)	0.28 ± 0.03 (16)	0.36 ± 0.04 (15)	0.41 ± 0.03 (19)	0.48 ± 0.06 (15)	0.42 ± 0.03 (13)	Two way ANOVA, sqrt	df=3,179, F=4.79 P=0.004	NS	NS

Note: Na: Sodium; Cl: chloride; K: potassium; Na:K: sodium to potassium ratio; Mg: Magnesium; Bicarb: bicarbonate; Ca: calcium; P: phosphorus.

Table 15. Haemolymph organic molecules in scallops from the 2014, 150 in^3 air gun experiment, based on exposure level (0-pass, 1-pass, 2-pass, 4-pass) and sample time (0, 14, 120 days post-exposure). All values are expressed as means ± SEM with sample sizes indicated in parentheses. Significant differences in response to exposure level are marked by different colours (dark grey=a, light grey=ab, white=b, black=c), in response to sample time by upper case letters and in response to the interaction between exposure and sample time by lower case letters.

Exposure	0-pass			1-pass			2-pass			4-pass			Test	Significance		
Time (days)	0	14	120	0	14	120	0	14	120	0	14	120		Exposure	Time	Interaction
TP ($g\ l^{-1}$)	0.82 ± 0.06 (20)	1.20 ± 0.00 (17)	0.91 ± 0.09 (15)	0.88 ± 0.07 (19)	0.94 ± 0.08 (15)	0.72 ± 0.06 (10)	0.69 ± 0.08 (18)	0.78 ± 0.06 (16)	0.75 ± 0.06 (15)	0.95 ± 0.08 (18)	0.87 ± 0.09 (14)	1.06 ± 0.10 (13)	rANOVA			df=6,178 Itr=2.579 P=0.02
Trig ($mmol\ l^{-1}$)	0.40 ± 0.17 (19)	0.19 ± 0.08 (17)	0.38 ± 0.16 (14)	0.08 ± 0.02 (17)	0.50 ± 0.21 (15)	0.25 ± 0.18 (10)	0.30 ± 0.11 (18)	0.21 ± 0.06 (16)	0.57 ± 0.27 (15)	0.90 ± 0.56 (18)	0.54 ± 0.28 (14)	0.31 ± 0.16 (13)	Two-way ANOVA	NS	NS	NS
Chol ($mmol\ l^{-1}$)	0.09 ± 0.01 (20)	0.11 ± 0.01 (17)	0.09 ± 0.01 (14)	0.10 ± 0.01 (19)	0.09 ± 0.01 (15)	0.10 ± 0.01 (10)	0.10 ± 0.00 (18)	0.11 ± 0.01 (16)	0.11 ± 0.01 (15)	0.13 ± 0.01 (19)	0.12 ± 0.01 (14)	0.11 ± 0.01 (13)	Two-way ANOVA	NS	NS	NS
Gluc ($mmol\ l^{-1}$)	0.16 ± 0.01 (20)	0.16 ± 0.03 (17)	0.19 ± 0.03 (15)	0.15 ± 0.01 (19)	0.14 ± 0.01 (15)	0.16 ± 0.02 (10)	0.15 ± 0.02 (18)	0.13 ± 0.02 (16)	0.13 ± 0.02 (15)	0.15 ± 0.02 (19)	0.09 ± 0.02 (14)	0.10 ± 0.02 (13)	Two-way ANOVA	df=3,180, F=5.37, P=0.01	NS	NS
Uric ($\mu mol\ l^{-1}$)	0.80 ± 0.14 (20)	0.29 ± 0.11 (17)	0.07 ± 0.07 (14)	0.37 ± 0.11 (18)	0.60 ± 0.21 (15)	0.40 ± 0.16 (10)	0.28 ± 0.16 (18)	0.50 ± 0.13 (16)	0.33 ± 0.13 (15)	0.47 ± 0.14 (18)	0.27 ± 0.12 (14)	0.15 ± 0.10 (13)	Two-way ANOVA	NS	NS	NS

Note: TP: total protein, Trig: triglyceride; Chol: cholesterol; Gluc: glucose; Uric: uric acid

Table 16. Haemolymph enzyme activities in scallops from the 2014, 150 in^3 air gun experiment, based on exposure level (0-pass, 1-pass, 2-pass, 4-pass) and sample time (0, 14, 120 days post-exposure). All values are expressed as means ± SEM with sample sizes indicated in parentheses.

Exposure	0-pass			1-pass			2-pass			4-pass			Test	Significance		
Time (days)	0	14	120	0	14	120	0	14	120	0	14	120		Exposure	Time	Interaction
AMY (U l^{-1})	14.00 ± 7.67 (20)	25.47 ± 12.77 (17)	85.53 ± 43.65 (14)	7.32 ± 4.44 (19)	10.87 ± 8.90 (15)	18.90 ± 16.92 (10)	49.67 ± 37.22 (18)	2.63 ± 0.83 (16)	4.53 ± 2.71 (15)	69.89 ± 31.65 (18)	4.93 ± 1.95 (14)	92.38 ± 85.69 (13)	Two-way ANOVA	NS	NS	NS
LIP (U l^{-1})	3.30 ± 0.51 (20)	4.94 ± 0.91 (17)	14.80 ± 7.08 (14)	4.58 ± 0.92 (18)	3.20 ± 0.33 (15)	4.10 ± 1.06 (10)	7.89 ± 4.21 (18)	3.00 ± 0.49 (16)	3.93 ± 0.46 (15)	15.95 ± 5.96 (18)	4.13 ± 0.46 (14)	12.46 ± 7.48 (13)	Two-way ANOVA	NS	NS	NS
ALP (U l^{-1})	0.70 ± 0.11 (20)	0.59 ± 0.12 (17)	0.93 ± 0.18 (14)	1.00 ± 0.08 (19)	0.47 ± 0.13 (15)	1.00 ± 0.26 (10)	0.89 ± 0.11 (18)	0.38 ± 0.13 (16)	0.40 ± 0.13 (15)	0.63 ± 0.14 (18)	0.67 ± 0.19 (14)	0.85 ± 0.15 (13)	Two-way ANOVA	NS	NS	NS
AST (U l^{-1})	0.05 ± 0.05 (20)	0.00 ± 0.00 (17)	0.00 ± 0.00 (14)	0.16 ± 0.16 (19)	0.07 ± 0.07 (15)	0.00 ± 0.00 (10)	0.06 ± 0.06 (18)	0.00 ± 0.00 (16)	0.13 ± 0.13 (15)	0.42 ± 0.27 (18)	0.20 ± 0.14 (14)	0.00 ± 0.00 (13)	Two-way ANOVA	NS	NS	NS
ALT (U l^{-1})	0.05 ± 0.05 (20)	0.06 ± 0.06 (17)	0.00 ± 0.00 (14)	0.05 ± 0.05 (19)	0.07 ± 0.07 (15)	0.00 ± 0.00 (10)	0.06 ± 0.06 (18)	0.00 ± 0.00 (16)	0.00 ± 0.00 (15)	0.21 ± 0.21 (18)	0.00 ± 0.00 (14)	0.08 ± 0.08 (13)	Two-way ANOVA	NS	NS	NS
GGT (U l^{-1})	0.05 ± 0.05 (20)	0.00 ± 0.00 (17)	0.00 ± 0.00 (14)	0.00 ± 0.00 (19)	0.07 ± 0.07 (15)	0.00 ± 0.00 (10)	0.06 ± 0.06 (18)	0.00 ± 0.00 (16)	0.00 ± 0.00 (15)	0.11 ± 0.11 (18)	0.07 ± 0.07 (14)	0.08 ± 0.08 (13)	Two-way ANOVA	NS	NS	NS

* AMY: amylase; LIP: lipase; ALP: alkaline phosphatase; AST: asparate aminotransferase; ALT: Alanine aminotransferase; GGT: gamma-glutamyl transferase.

Behaviour

Analysis of video recording before, during and after air gun runs (henceforth pre-, intra- and post-exposure) was used to quantify three components of scallop behaviour: classic behaviours, non-classic behaviours and tentacle extension. A summary of behavioural observations is given in Table 17.

A novel, non-classic behaviour, best described as a velar flinch, in which the velum was rapidly retracted while the tentacles and the valves maintained a constant position, was observed exclusively in response to air gun signals during intra-exposure time periods. The behaviour was observed in response to air gun signals at a maximum range of approximately 350 m, and continued to occur as the vessel moved closer. It was commonly observed just prior to the audible air gun signal on the video and the behaviour differed from the classic "cough" or "close" in that the position of the upper valve was maintained in its "normal" state, i.e. it was neither adducted nor abducted relative to where it was held in a resting state prior to the air gun signal. Similarly, the mantle and the tentacles also maintained their natural position and were not retracted. Rather, the velum was rapidly drawn in, as if being sucked in, and then returned to position, with the whole behaviour lasting less than 1 second. This was the only observed behaviour to be categorised as non-classic and occurred only in exposed scallops.

Exposed scallops exhibited the highest rate of non-classic behaviour during the exposure period and a lower rate subsequently. The two and four pass exposures had significantly higher non-classic rates than the single exposure treatment ($p=0.002$). During the exposure the classic behaviour rate was significantly lower ($p<0.001$) across all treatments, however after the exposure there was no significant difference ($p=0.14$).

In 2014 the same scallops were used as the numbers of passes was increased, consequently pre-exposure scallops for the two and four pass treatments had already been exposed. The non-classic behaviour was not observed during the two and four pass pre-exposure periods and the rate of classic behaviour in the pre-exposure period did not differ significantly ($p=0.38$). This indicates that the delay between passes was sufficient for the scallops to cease their behavioural anomaly.

Tentacle extension behaviour in the 2014 and 2015 experiments was also compared between pre-, intra- and post-exposure time periods (Fig. 45). Cursory examination of the data suggested the lack of any consistent trends with the level of exposure and the high variability of behaviour in all treatments and times indicated that the natural behavioural variability exceeds any possible underlying behavioural changes in this experiment. This was confirmed by a multinomial regression which found no significant relationships.

Qualitative observations of the video were made to evaluate the occurrence of energetically expensive behaviours, namely extended periods of swimming or valve closure. In the 2014 experiment, swimming was observed in 2 individuals, one 1-pass individual swam once prior to the control run and one 4-pass individual swam three times, twice prior to air gun exposure and once during exposure. All 4 occurrences were brief (<5 s) and appeared to be in response to the movement of another scallop or to adjust positioning. No scallops were observed to swim in the 2015 experiment. In both 2014 and 2015 experiments there was one occurrence each of an

individual remaining closed for the entire duration of the experiment, in a 1-pass individual in the 2014 experiment and a 4-pass individual in the 2015 experiment.

Table 17. Behavioural observations in scallops. Given values indicate the total number of classic and non-classic behaviours observed by treatment during pre-, intra- and post-exposure periods during seismic air gun runs during the winter 2014, 150 in^3 air gun experiment and then summer 2015, 150 in^3 air gun experiment. Sample size, in terms of the number of individuals observed, is given in parentheses for each category. Variations in sample size between exposure periods resulted from inability to observe individual scallops during a particular exposure period. The observations of non-classic behaviour are shown in bold.

	Pre-exposure			Intra-exposure			Post-exposure		
Treatment	Classic	Non-classic	Time	Classic	Non-classic	Time	Classic	Non-classic	Time
2014									
0	40 (n=19)	0 (n=19)	4h48m6s	36 (n=18)	0 (n=18)	5h11m36s	27 (n=19)	0 (n=19)	4h5m10s
1	23 (n=16)	0 (n=16)	3h18m12s	21 (n=16)	**29** (n=16)	7h36m48s	13 (n=14)	2 (n=14)	3h31m38s
2	3 (n=8)	0 (n=8)	2h23m11s	0 (n=8)	**35** (n=8)	1h54m29s	6 (n=8)	0 (n=8)	2h7m3s
4	21 (n=8)	0 (n=8)	3h44m16s	4 (n=8)	**22** (n=8)	1h36m6s	11 (n=8)	0 (n=8)	2h3m46s
2015									
0	24 (n=12)	0 (n=12)	1h42m36s	22 (n=12)	0 (n=12)	3h16m0s	16 (n=12)	0 (n=12)	1h44m28s
4	3 (n=7)	0 (n=7)	49m51s	10 (n=7)	**21** (n=7)	1h35m59s	2 (n=7)	**1**	2h12m5s (n=7)

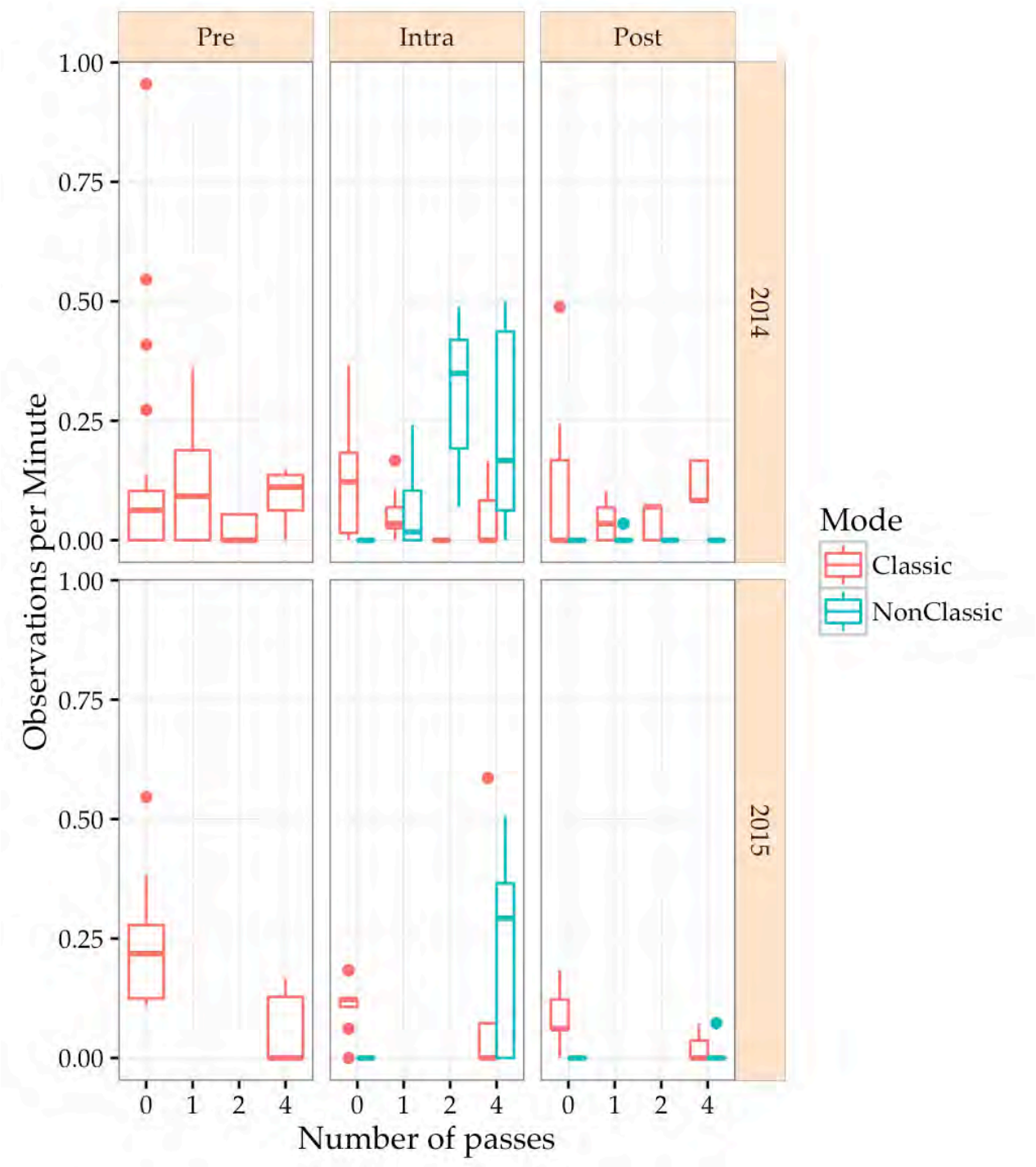


Figure 44. The rate of scallop behaviour for each exposure during pre-, intra- and post-exposure time periods observed from video recordings of air gun exposure during winter 2014, 150 in^3 air gun experiment (top) and summer 2015, 150 in^3 air gun experiment (bottom). Non-classic behaviours were only observed in exposed scallops during or after exposure.

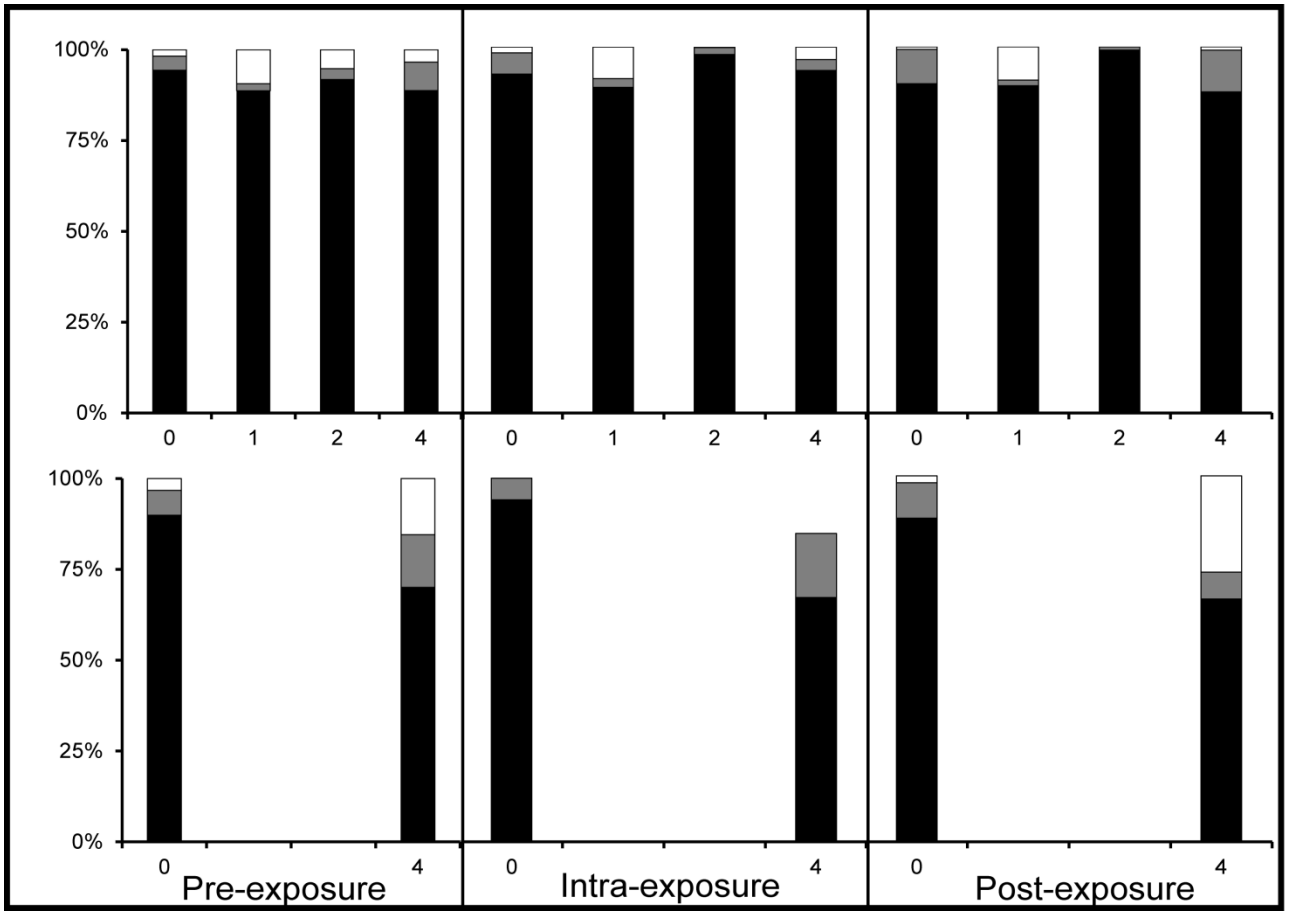


Figure 45. Scallop tentacle extension during pre-, intra- and post-exposure time periods observed from video recordings of air gun exposure during winter 2014, 150 in^3 air gun experiment (top) and summer 2015, 150 in^3 air gun experiment (bottom), expressed as percentages of total observation time. Tentacle state is indicated by colour, with extension represented by black, partial extension represented by grey and retraction represented by white. Column segments labelled with differing letters were significantly different.

Recessing and righting reflexes

For the 2013 experiment, the median time-to-recessing (Fig. 46A) for 0-pass, 1-pass, 2-pass and 4-pass scallops were 60, 36, 36 and 24 h, respectively, and the curves were found to be significantly different ($\chi^2(3)=18.06$, N=131, P<0.001). Multiple comparison with Bonferroni correction showed significant differences in survival time between 0-pass and 4-pass scallops and between 1-pass and 4-pass scallops. For the 2014 experiment, the recessing test was performed twice: immediately after exposure as in the 2013 experiment and again just prior to the day 120 sampling point. For the first test (Fig. 46B), median time to recessing was 48 h for 0-pass, 36 h for 1-pass, 42 h for 2-pass and 36h for 4-pass and analysis again showed a significant difference in survival curves ($\chi^2(3)=16.33$, N=146, P<0.001), with 0-pass scallops significantly slower to recess than 2-pass and 4-pass treatments. In the second recessing test for the 2014 experiment (Fig. 46C), conducted prior to day 120, median time to recessing was markedly longer than previous, with 72 h for 0-pass, 45 h for 1-pass, 48 h for 2-pass and 54 h for 4 pass. Recessing curves were again significantly different ($\chi2(3)=8.66$, N=55, P=0.034), and multiple comparison showing that 0-pass scallops were significantly slower than 4-pass scallops.

For the 2015 experiment (Fig. 46D), the recessing test was only performed immediately after air gun exposure due to mortality of all scallops prior to the 120 day sample point. Median recessing time was 60.5 h for 0-pass scallops and 45 h for 4-pass scallops and, again, the survival curves were significantly different ($\chi^2(1)=13.30$, N=65, $P<0.001$).

The righting test conducted in the 2015 experiment (Fig. 47) showed that that 0-pass scallops, which had a mean righting time of 516 s, righted themselves significantly faster than 4-pass scallops ($\chi^2(1)=4.437$, N=70, $P=0.036$), which had a mean righting time of 710 s.

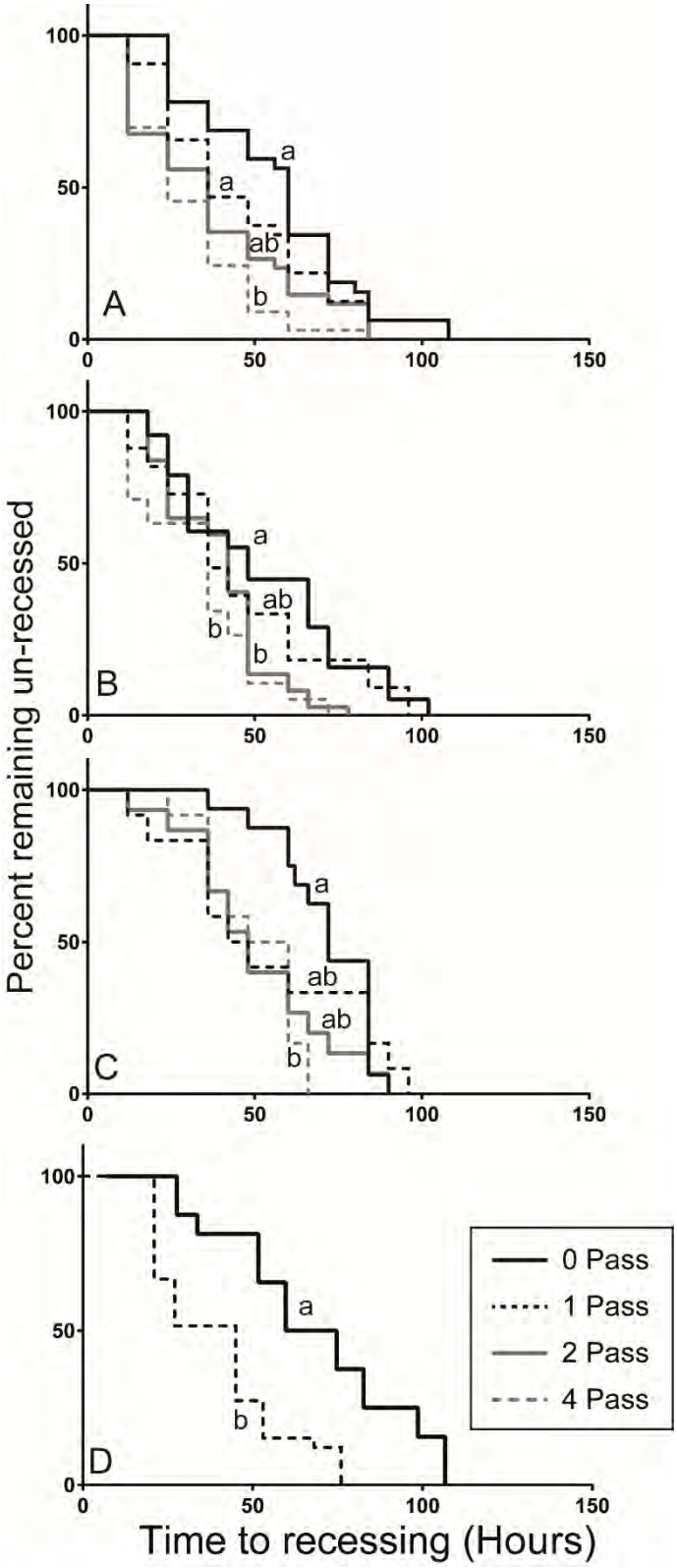


Figure 46. Kaplan-Maier survival curves depicting the results of the recessing reflex test for the a) winter 2013, 45in^3 air gun experiment; b) winter 2014, 150 in^3 air gun experiment at day 14; c) winter 2014, 150 in^3 air gun experiment at day 120 and d) summer 2015, 150 in^3 air gun experiment. Within each experiment, significantly different survival curves as determined using logrank (Mantel-Cox) tests are indicated by differing letters.

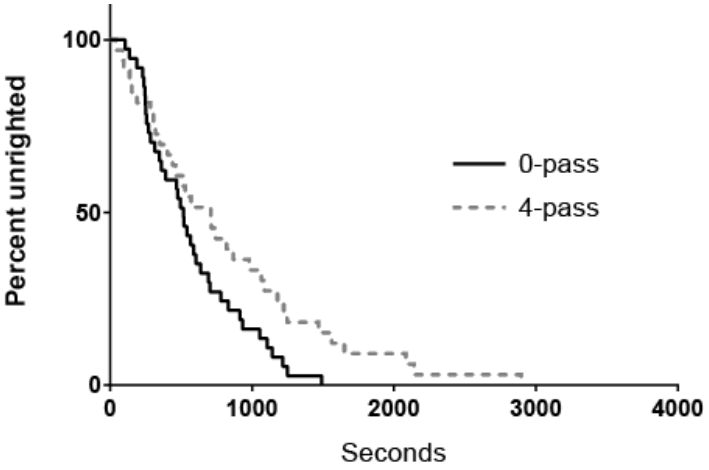


Figure 47. Kaplan-Maier survival curves depicting the results of the righting reflex test, which was conducted at day 14 of the summer 2015, 150 in^3 air gun experiment.

Condition indices

From the measurements recorded when scallops were sampled, 5 condition indices (Fig. 48) were compared to determine whether seismic exposure had any effect. The first index, mass-to-length, showed that in the 2013 experiment, the number of air gun passes did not have a significant effect ($F(3,189)=2.387$, $P=0.07$) but that sample time had a significant effect ($F(2,189)=92.373$, $P<0.001$). There was no significant interaction between the two factors ($F(6,189)=1.698$, $P=0.13$). Post hoc analysis showed that all four treatments had significantly reduced mass-to-length ratios at day 120, with a 31% reduction relative to day 0 and a 29% reduction relative to day 14. In the 2014 experiment, air gun exposure was not a significant factor ($F(3,171)=0.941$, $P=0.42$), sample time was a significant factor ($F(2,171)=9.449$, $P<0.001$), and the interaction between the two was not significant ($F(6,171)=1.906$, $P=0.082$). Post-hoc analysis of sample time indicated that day 120 treatments showed a significantly reduced mass-to-length ratio, with a reduction of approximately 8% relative to values on both day 0 and day 14. In the 2015 experiment, there was no difference at day 14 ($t(26.75)=0.59$, $P=0.56$).

The second index, mass-to-volume, showed no significant differences for either the number of air gun passes ($F(3,189)=1.161$, $P=0.33$) or sample time ($F(2,189)=1.485$, $P=0.229$) or the interaction of the two factors ($F(6,189)=0.409$, $P=0.87$) in the 2013 experiment. Similarly, in the 2014 experiment, air gun exposure was not a significant factor ($F(3,171)=0.024$, $P=0.99$), sample time was not a significant factor ($F(2,171)=0.789$, $P=0.46$) and there was no significant interaction between the factors ($F(6,171)=1.810$, $P=0.10$). For the 2015 experiment, there was no significant difference found at day 14 ($t(27.34)=0.65$, $P=0.52$).

The third index, relative tissue mass, showed a significant interaction between air gun exposure and sample time ($F(6,173)=5.603$, $P<0.001$) for the 2013 experiment. At day 0, scallops from the 1-pass treatment showed a significantly greater relative tissue mass than scallops from the 2-pass (11% greater) and 4-pass treatments (8% greater). At day 14, there were no differences between treatments as all four showed a level similar to that of day 0. At day 120, all four treatments again showed a similar relative tissue mass at a level significantly reduced, by approximately 30%,

relative to both day 0 and day 14. In the 2014 experiment there was again a significant interaction between air gun exposure and sample time ($F(6,189)=2.440$, $P=0.027$). There were no significant differences between treatments at day 0 in this experiment, though at day 14, 0-pass and 1-pass treatments showed relative tissue mass values approximately 10% lower than those of 2-pass and 4-pass treatments, a difference that was significant. At day 120, there were no differences in relative tissue mass between treatments, but all treatments experienced significant decreases relative to day 0 and day 14, with 0-pass and 1-pass scallops showing a decrease of 8% and 5%, respectively, relative to day 0 and 4% and 3%, respectively, relative to day 14 and 2-pass and 4-pass scallops showing no decrease and a 12% decrease, respectively, relative to day 0 and a 7% and 15% decrease, respectively, relative to day 14.

The 2014 experiment followed a similar trend, with 0-pass, 2-pass and 4-pass treatments showing significant differences between sample Day 0 and Day 120 and between Day 0 and Day 14 for 2-pass and 4-pass treatments. Although the 2014 experiment did not show any significant differences at Day 0 like the previous experiment, at Day 14 both 0-pass and 1-pass scallops had a significantly lower relative tissue mass than 2-pass and 4-pass treatments and at Day 120, 2-pass scallops had a significantly greater index than 4-pass scallops. For the 2015 experiment, no difference ($t(33.13)=-0.90$, $P=0.38$) was found between 0- and 4-pass scallops at Day 14 using Welch t-test.

For the fourth condition index, percent adductor mass, in the 2013 experiment, air gun exposure was not a significant factor ($F(3,189)=1.151$,$P=0.33$), sample time was a significant factor ($F(2,189)=145.54$, $P<0.001$) and the interaction between the two factors was not significant ($F(6,189)=0.762$, $P=0.60$). Percent adductor mass was found to significantly increase in all treatments at the day 120 sample relative to that of day 0 by an average of 55% and to that of day 14 by an average of 54%. The 2014 experiment had a similar result for percent adductor mass, as exposure was not a significant factor ($F(3,181)=0.505$, $P=0.68$), sample time was a significant factor ($F(2,181)=7.719$, $P<0.001$) and there was no significant interaction between the factors ($F(6,181)=0.627$, $P=0.71$), with post hoc testing showing a significant reduction of 9% in percent adductor mass at the 120 day sample point relative to day 0 and a significant reduction of 6% compared to the day 14 sample point. In the 2015 experiment, Welch t-test showed a significant difference between 0-pass and 4-pass treatments, as the latter showed an 8.5% lower percent adductor mass ($t(30.36)=-2.26$, $P=0.032$)

Relative adductor mass, the fifth condition index, showed a significant response to sample time ($F(2,189)=302.865$, $P<0.001$) but not to air gun exposure ($F(3,189)=1.151$, $P=0.33$) or the interaction of the two factors ($F(6,189)=1.276$, $P=0.28$). The difference in this index was a significant, 2-fold increase on average in all four treatment levels at day 120 relative to days 0 and 14. In the 2014 experiment, neither exposure ($F(3,181)=1.083$, $P=0.36$) nor sample time ($F(2,181)=0.918$, $P=0.40$) were significant factors. In the 2015 experiment, Welch t-test showed a significant difference between 0-pass and 4-pass treatments for relative adductor mass ($t(32.47)=-2.06$, $P=0.048$), with 0-pass scallops showing an 8% greater relative adductor mass value.

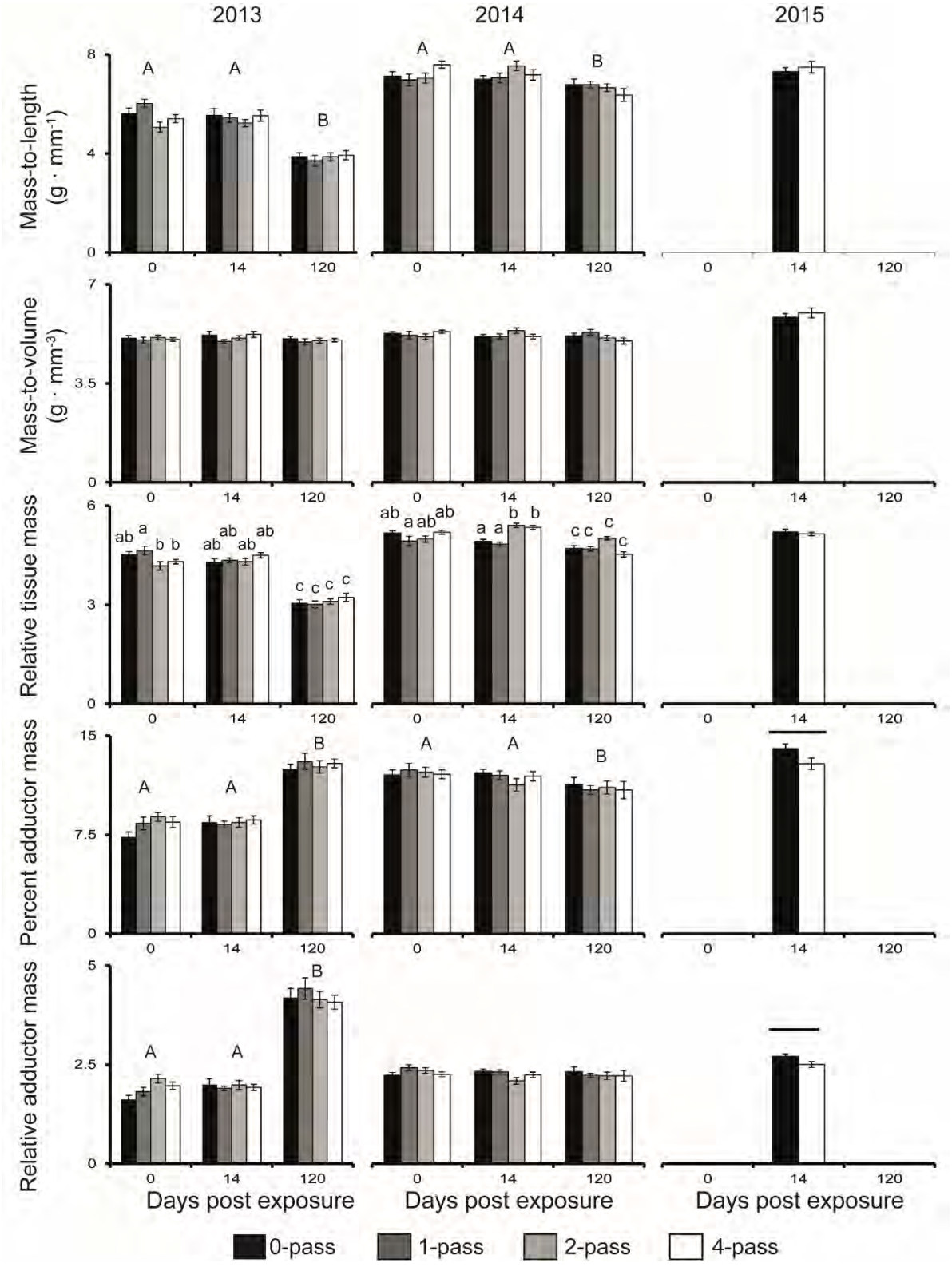


Figure 48. Mean (± SEM) values of condition indices for scallops from the winter 2013, 45 in^3 air gun experiment; the winter 2014, 150 in^3 air gun experiment and the summer 2015, 150 in^3 air gun experiment. For each experiment, significant differences in response to sample time are indicated with upper-case letters, in response to exposure are indicated with horizontal bars and interaction between the factors are indicated with lower-case letters, as determined using two-way ANOVAs (2013, 2014). Significant differences in response to exposure as determined by Welch two-sample t-test (2015) are also indicated by a horizontal bar.

Discussion

Seismic exposure

The maximum measured exposures observed in any experiment from the sea noise loggers were 189 dB re 1µPa^2·s SEL, 211 dB re 1µPa peak-peak and the maximum peak magnitude of ground acceleration immediately following the waterborne arrival of 68 ms^{-2}, although this was an outlier and levels were more likely in the range 15-20 dB re 1ms^{-2} (Figure 16). We had one to four sea noise loggers deployed per experiment with the experimental animals spaced out over 30-250 m range. Thus not all animals would have experienced sound levels similar to the maximum values measured, rather some may have experienced levels of this magnitude but most would have experienced lower exposures. In order to deal with this we calculated the received level of every air gun, at every animal's cage or pot, then presented statistics of exposures received (Table 5 and Table 7). Thus 'averages' of exposure received for each pass or multiple passes of the air gun have been used to define exposures. Maximum calculated sound exposure level (SEL) and geophone values reported here from any single experiment or pass, compared with similar measurements available from commercial seismic sources are presented in Table 18.

Table 18. Maximum calculated air gun sound exposure levels (SEL dB re 1µPa^2·s), cumulative sound exposure level (SEL$_{cum}$ dB re 1µPa^2·s) and maximum peak magnitude of seabed acceleration (ms^{-2} and dB re 1ms^{-2}) from single passes of the air gun measured here for the 45 and 150 in^3 chambers (using the fitted curve trend), compared with similar measures from air gun arrays. Ranges refer to closest points of approach distance, with this being the closest cage in these experiments. na = not available.

Reference	Source	Min range (m)	max SEL (dB re 1µPa^2·s)	max SEL$_{cum}$ (dB re 1µPa^2·s)	max Geo. (ms^{-2} / dB / range in m)
these measures	45 in^3 single gun single pass cpa	6	181	189	37 / 15.7 / 6
these measures	150 in^3 single gun single pass cpa	6	188	188-192	38 / 15.8 / 6
Tashmukhambetov *et al.* (2008)	3590 in^3 array in 990 m water depth, receiver 250 m off seabed	740	178	187	na
McCauley & Gavrilov (2010)	3040 in^3 array in 152 m depth, source overhead, receiver on seabed, single pass cpa	152	178	189	na
McCauley & Gavrilov (2010)	2130 in^3 array in 152 m depth, source overhead, receiver on seabed, single pass cpa	152	174	188	na
McCauley (unpublished)	3130 in^3 array in 36 m water, receiver on seabed, single pass cpa	477	172	190	6.2 / 8.0 / 477

A common criticism of animal exposure experiments with air gun sources is that they do not represent "real" seismic sources or that the exposure either exceeds or is lower than that of a "real" seismic source. To address this, modelling of a commercial seismic sources passing over a receiver was carried out to define how the exposures presented here compared with those from a commercial source (summarised in Figure 18). It needs to be remembered in comparing exposures that curves presented for experimental and commercial surveys are based on statistics. There is always variability in commercial seismic air gun signals propagating horizontally (2-4 dB and 1-3 dB standard deviation at a given range within a survey and shot-shot, respectively, McCauley *et al*. 2016) and there are considerable differences between different seismic survey configurations (3-12 dB standard deviation at any range, McCauley *et al*. 2016). Thus comparisons given here should be considered an approximate range over which a commercial source would compare with the experimental exposures.

For the single pass lobster experiments (exposures in Table 5) sound exposure level is considered the most pertinent stimuli as the lobster were reasonably de-coupled from the substrate by their legs. The maximum single shot exposures experienced in experiments were equivalent to the nearest sail line of a 3065 source (mean of measured transmission of a 3040 and 3090 in^3 source, McCauley *et al.* 2016) at 100-200 m (range due to different estimated maximum values during different experiments). The median cumulative exposures experienced during experiments were equivalent to a set of five seismic lines with the nearest sail line at 200-500 m range. Thus the exposures experienced during lobster experiments can be considered to be equivalent to a commercial ~ 3100 in^3 seismic source passing within 100-500 m range adjacent the lobsters.

For the scallop experiments three exposure regimes were used plus two air gun source volumes. Each experiment had zero (control), one, two or four passes of the experimental source. Impacts observed were dose dependant and impacts occurred for all exposure regimes. For scallops the ground motion is important as they are directly coupled to the ground. It was noted in the results that: 1) ground roll was well correlated with sound exposure level; 2) that when accounting for cumulative ground roll and time of exposure in calculations by deriving ground roll measures in a similar fashion to SEL calculations, the maximum single shot magnitude of ground roll correlated well with the cumulative or SEL type ground roll calculations; and 3) it was believed a large fraction of the ground roll observed derived from the transfer of energy from the waterborne particle acceleration into the ground (thus waterborne SEL can be expected to partly define ground roll measures). Thus as there are no definitions of how to define ground roll for biological impacts we have chosen to use the simplest measure, maximum magnitude of the three axis ground roll for a single shot, to define ground roll when comparing our experimental exposures with those estimated for a commercial seismic source. To compare experimental exposures with those estimated from a large commercial array the experimental exposure values given in Table 7 were used with estimated exposures from a commercial source as shown on Figure 18 to give the nearest sail line bounds to match that exposure. The resulting values for all combinations of experiment, SEL, cumulative SEL and ground roll (in linear units) are given in Table 19.

Based on values given in Table 19 we can get an estimate of the approximate closest sail line for a commercial array to give equivalent maximum SEL, median cumulative SEL, and ground roll, as the outlying range for each experimental pass. Using values in Table 19 for scallops the single pass exposures were equivalent to a large commercial array passing with the closest sail line at 114-875 m range, the second pass at 114-500 m range and the third pass at 115-275 m range.

Table 19: Comparison of experimental scallop exposures and estimated equivalent range of hypothetical seismic survey, giving experimental regime, estimated exposure received during that experiment and the estimated range this exposure occurred from a commercial array as displayed on Figure 18 (Range$_E$ in m). The experiments are labelled by year (2013, 2014 and 2015) with 1, 2 and 4 passes within an experiment, with cumulative SEL from multiple passes indicated by Pass 1+2 and Pass 1+2+4. The estimated exposures of: maximum SEL experience; median cumulative SEL; and maximum magnitude of ground roll acceleration. Units are: SEL and SEL$_{cum}$ dB re 1µPa^2·s, and ground roll (GR) as ms^{-2}.

Experimental regime	Max SEL	Range$_E$ (m)	Median SEL$_{cum}$	Range$_E$ (m)	Max GR (linear)	Range$_E$ (m)
2013 Pass 1	181	250	189	725	37.2	114
2013 Pass 2	181	250	191	500	37.3	114
2013 Pass 4	181	250	194	275	37.6	118
2013 Pass 1+2			196	200		
2013 Pass 1+2+4			197	175		
2014 Pass 1	187	150	192	400	31.6	129
2014 Pass 2	188	150	194	275	35.4	117
2014 Pass 4	188	150	198	200	36.4	120
2014 Pass 1+2			196	175		
2014 Pass 1+2+4			200	100		
2015 Pass 1	188	150	188	875	35.5	117
2015 Pass 2	188	150	193	325	36.6	115
2015 Pass 4	188	150	196	175	36.6	115
2015 Pass 1+2			194	275		
2015 Pass 1+2+4			198	125		

The single air gun was used in open water field conditions to emulate a larger commercial seismic source comprised of an array of air guns operating at short range (within approximately a km, or termed the near-field here). The single air gun used was not a large commercial air gun seismic source but with the 150 in^3 chamber was a modest size individual air gun. In air gun arrays individual air guns are commonly clustered where two or three guns are co-located in close proximity (< 1 m) to essentially form a larger gun. These gun clusters are normally of 200-500 in^3 capacity so it is common for 'single' larger air gun sources (compared to the 150 in^3 gun used here) to be used in air gun arrays. In terms of sound exposure level values and the ground excitation produced, the exposures measured from the single air gun during experiments here were shown to be comparable to a large commercial source passing within a few hundred metre range. It should be noted that commercial air gun

arrays vary enormously in their size and spatial configurations between different contractors and for different tasks, thus there will be high variability in exposures produced by commercial air gun arrays within one km. The modelling suggests a large commercial seismic source at short range (< 100 m for scallops, < 200 m for lobsters) operating in 30-100 m water depth would produce higher sound exposure levels and ground motion than were produced in the experiments here (Figure 18).

The exposure strategy employed here was to define if significant biological impacts arose from short range exposure equivalent to passes of a commercial air gun array. To this end we had limited options in how air gun signals were presented, as we had a limited number of experiments to work with. We elected to do pass-bys of the continually operating air gun, as would be experienced during actual seismic surveys and to combine this with highly detailed and systematic physiological and behavioural, time-series sampling. Thus we had limited power available to ascertain threshold levels at which impacts would or would not occur as the minimum threshold we had available was one pass of the air gun for the lobster and four passes for the scallop experiments. For the scallop experiments where we sampled at multiple passes (0, 1, 2 and 4 passes) we did see differences in the relationship between number of passes and long term mortality indicating that repeated passes of a seismic survey at levels experienced here did cause additive effects. But we have not been able to ascertain at what threshold impacts began to be observed due to the experimental power available which was set by budget, resources available and that we had almost no starting base to begin with to assess what impacts and impact mechanisms would actually occur.

Lobsters

Mortality

There were no mortalities nor any observation of moribundity in lobsters exposed to seismic air gun signals. However, a number of subtle, sub-lethal impacts were observed over the course of this study.

Reflex behaviour and statocyst morphology

Two reflex behaviours, tail tonicity and righting, were assessed based on their ease of observation and their use in lobster fishery industries in the process of grading animals for likelihood of survival (Paterson *et al.* 2005). Tail tonicity, as measured through relative tail gape, is a simple reflex with inability to maintain tail tonicity considered symptomatic of fatigue (Spanoghe & Bourne 1997). In the three experiments conducted during the winter, lobsters exposed to air gun signals did not show a difference in tail gape. Comparing between these experiments shows that relative tail gape tended to decrease, indicating improving tail tonicity, over the course of the sample points in all three winter experiments, with the lowest gape observed at the 120 day sample point in all cases. This improvement is likely a result of increased nutritional condition post-moult and egg extrusion, which is supported by the marked increase in tail gape at day 365 relative to day 120 in the 2014 low pressure experiment.

In the summer experiment, exposure resulted in a significantly increased tail gape, indicating a reduced capacity for tail extension over 14 days following exposure. Warmer water temperatures in summer have a well-documented exacerbating effect on stress response in crustaceans, resulting in elevated mortality rates in a range of taxa (Goodrick *et al.* 1993; Paterson *et al.* 1993; Spanoghe & Bourne 1997; Castro *et al.* 2003). This has been attributed to colder water depressing activity (Goodrick *et al.* 1993; Paterson *et al.* 1993), metabolic rate (Spanoghe & Bourne 1997) and providing an anaesthetic effect (Paterson 1993), thus minimising the impact of stressors. Although the warmer temperatures in the 2015 summer experiment conditions may explain why air gun exposure resulted in a greater impact compared to the winter experiments, given that reduced tail tonicity has been attributed to fatigue, it is surprising that this difference would persist to 14 days after exposure. A study investigating recovery times after swimming to exhaustion in juvenile spiny lobster *Sagmariasus verrauxi* found that lobsters returned to basal metabolic rates within 14 hours of recovery (Jensen *et al.* 2013), suggesting that if reduced tail tonicity in exposed lobsters resulted from fatigue, the effect should not have lasted to day 2 post-exposure, much less day 14. Further research will be necessary to determine the cause of this disruption. Longer term sampling would be useful to determine how long this disrupted reflex might persist and measurements of metabolic rate would provide the data necessary to determine whether fatigue recovery was a factor. Neural control of the tail could be another line of investigation warranting investigation. Although a slightly (*ca.* 3.5% of body length) compromised ability to maintain the tail erect might seem minor, the disruption of such a simple reflex may underlie the disruption of more complex behaviours, including feeding, predator avoidance, locomotion and social behaviours (Vermeer 1983).

Indeed, the lobster righting response, a complex reflex requiring neurological control and muscle coordination (Stoner 2009), showed a significant response to exposure in the winter 2013, 45 in^3 standard pressure experiment, the winter 2014, 150 in^3 low pressure experiment and the summer 2015 150 in^3 standard pressure experiment. To understand the increased righting time observed in exposed lobsters, the morphology of the principle balance sensory organ, the statocyst, was investigated. Statocysts are commonly found in aquatic invertebrates, including bivalves, cnidarians, echinoderms, cephalopods and crustaceans (Sekiguchi & Terazawa 1997), and in lobsters, the statocyst is a paired organ located on the basal segment of the antennules with an opening on the dorsal surface.

The statocyst of *J. edwardsii* has been previously described by Sekiguchi & Terazaw (1997), however, their description was limited to the puerulus life stage. Several notable differences were observed in the adults examined in the current study, as the capsule was fluid filled and lined with sensory hair cells. These sensory hairs, or setae, were found to be in contact with an accretion of numerous smaller particles, called statoconia (where a single mass is present termed a statolith). Gravity acts upon the statoconia or statolith, affecting the angle of flexion of the sensory hairs, acting as a stimulus which confers the ability to sense and adjust body position. Cohen (1955; 1960) described two types of hairs in the American lobster *Homarus americanus*, statolith hairs and thread hairs, the former of which were found to be in contact with the statolith and function as position receptors, and the latter of which project into the

fluid of the statocyst and sense acceleration as the fluid of the statocyst circulates in response to positional changes.

Based solely on morphological observation and an assumption of roughly analogous function between the hairs of the morphologically similar *J. edwardsii* and *H. americanus* statocysts, the hairs contained in zones 3 and 4 in *Jasus edwardsii* appear to be statolith hairs and the hairs in zone 2 appear to be matt hairs (Cohen 1955; 1960; Patton & Grove 1992). Given that the setae in zone 1 lacked contact with the statolith, were densely arranged and do not fit the morphological description of the slender thread hairs, these hairs may function either as non-sensory guard hairs, considering their location at the entrance of the statocyst, or as detectors of angular acceleration as in the similarly anteriorly located setae described by Hertwig *et al.* (1991) and Finley & Macmillan (2000).

The damage observed in lobsters occurred predominantly in zones 3 and 4, in the assumed statolith hairs, suggesting the statolith/statocina may have been driven harshly by the seismic air gun signals, causing the hair to be severed at the "casque," the flexible joint where the hair extends from the pore (Patton & Grove 1992). As would be predicted from damage to the hairs responsible for position reception, damage to the statocyst had a significant effect on righting in the winter 2013, 45 in^3 standard pressure, winter 2014, 150 in^3 low pressure and summer 2015, 150 in^3 standard pressure experiments, with greater amounts of damage indicative of a slowed righting reflex. The relative lack of damage to setae in zone 1 suggests that sensation of acceleration should be unaffected.

The ecological impacts of reduced body position sensation and corresponding impaired righting ability are not entirely clear. The impact of air gun signals on the lobster statocyst is clearly not as exquisite as that reported in cephalopods, in which the statocyst demonstrated severe, temporally progressive lesions in the sensory hair cells (André *et al.* 2011; Solé *et al.* 2012; Solé *et al.* 2013). However, there is likely some impact on the ability of an exposed lobster to function in the wild. Lobsters use input from the statocysts, leg proprioception receptors and eyes in conjunction to identify and modulate their position (Neil 1985), and removal of one of these inputs forces a greater reliance on the others (Schöne *et al.* 1983). This input controls a range of behaviours in lobsters, including the movement of the eyes, movement of the antennae and coordination of the tail (Schöne *et al.* 1983; Neil 1985; Newland & Neil 1987). Indeed, experiments show that the statocyst provides input during the tail flip escape response that allows lobsters to mediate their upright position and that removal of the statocyst entirely compromises the ability to modulate swimming to correct their body position (Newland & Neil 1990) and to return to the substrate in an upright position (Newland & Neil 1987), a posture necessary to initiate any further escape responses. Following moulting, when the statocyst is shed with the rest of the carapace, lobsters have been suggested to have impaired statocyst function due to the species specific pace of development of the sensory setae (Cohen 1960), which may play a role in the observation of reclusiveness and inactivity during and immediately following moulting (Lipcius & Herrnkind 1982; Kelly *et al.* 1999), despite retaining agility and demonstrating the capability of performing intense and coordinated activity in laboratory settings (Lipcius & Herrnkind 1982). These behavioural modifications may be an adaptive response to the decreased

sensory input of the maturing statocyst setae and the corresponding reduction of the ability to both acquire prey and avoid becoming prey.

A second consideration of the ecological impact is the ability, or lack thereof, to regrow or replace damaged hairs. Schmitz (1992) and Finley & Macmillan (2000) reported that in the crayfish *Orconectes limosus* and *Cherax destructor*, respectively, statocyst size and the number of setae increased linearly with carapace length. If the statocyst can continue to grow and the setae proliferate normally following the damage incurred from air gun signals, there is a potential for the damage, or at least any loss of sensory input, to be ameliorated. However, it must be noted that in the present study, the damage persisted until day 365 post-exposure, a period over which all individuals had moulted. As the statocyst is part of the cuticle, it is shed during the moult process and the lack of any hair regeneration indicates the damage is potentially permanent, as are the effects, as evidenced through the corresponding persistence of righting reflex impairment.

In contrast to the clear relationship between exposure and statocyst damage from the other experiments, results from the lobsters used in winter 2014, 150 in^3 standard pressure experiment were markedly different. The control treatment of lobsters in this experiment, which were collected from the Crayfish Point Reserve in Taroona, showed a level of statocyst damage similar to that of exposed treatments from the air gun experiments. Air gun exposure did not result in additional setae damage in the exposed treatment relative to controls and there were no significant differences in righting time in these lobsters at any of the sample times. In considering these results, it is important to note some differences between the collection site of the lobsters used in the winter 2013, 45 in^3 standard pressure, winter 2014, 150 in^3 low pressure and summer 2015, 150 in^3 standard pressure experiments versus that of the winter 2014, 150 in^3 standard pressure lobsters (Fig. 49). For the former, lobsters were collected from depths between 40 and 60 m at sites off the southern coast of Tasmania, a relatively remote area with little marine traffic apart from fishing vessels. For the latter, lobsters were collected from a shallow water (>15 m) site within the Crayfish Point Reserve, off Taroona. This site lies in the mouth of the Derwent River in close proximity to the major shipping lane into the city of Hobart, which sees a comparatively greater volume of marine traffic from both recreational vessels and large commercial vessels, such as container ships and cruise ships, which generate constant, high energy sound output, with source levels 192 dB re 1 uPa @ 1 m reported for container ships (Hildebrand 2009). Noise loggers placed at the two collection sites enabled quantification of the differences in the soundscapes of the two sites, showing that lobsters collected from the site off the southern coast were exposed to slightly higher baseline ambient noise level due to the rougher seas and unprotected nature of the site, but that the Taroona site showed substantial levels of sporadic, intense anthropogenic noise from large vessels and constant, low-frequency noise of a lower intensity from an unidentified anthropogenic source possibly associated with localised pumping systems.

Based on these sound exposure levels, it is believed that the damage observed in the control lobsters collected from the Taroona site resulted from environmental exposure to nearby noise, most likely of anthropogenic origin. An investigation of the statocyst of the American lobster *Homarus americanus* by Patton & Grove (1992) demonstrated how the signals from

statocyst hair cells are interpreted to coordinate the muscular reaction to changes in body position, such as pitch or roll, and provides insight into the relationship between the hair cell damage and righting results. Their work showed that the irregular shape of the statocyst resulted in variability in the number and distribution of hair cells touching the statolith as the lobster changed its body orientation. Their finding indicates that lobsters compensate for this by summing the inputs from many hairs to formulate a determination of body position, with hair cells demonstrating the capacity for an adaptive response to sensory input as the irregularity in the shape of the statolith results in a haphazard variation in hair angle, forcing the lobster to "learn" to interpret the signal from the hair as the statocyst changes position following violent movements like the tail flip escape response or after the statolith is replaced following moulting. While Patton and Grove (1992) described the evolution of this system as "clumsy and metabolically expensive," this adaptation to irregularities in the statolith may provide lobsters with a degree of resilience to the loss of hair cells, with the the summative nature of the sensory response providing a redundancy mechanism, allowing the lobsters to adapt to the loss of hair cells through the recruitment of other nearby hairs as they do when the statolith is reoriented or replaced.

A number of questions remain regarding the results from this study. While environmental exposure to intense noise explains the damage observed in the control lobsters from Taroona, it does not explain the lack of damage incurred in the exposed lobsters from that site. If the adaptive capacity of the hair cells allows for undamaged hairs to fill in for damaged hairs, subsequent exposure should be expected to result in further damage. However, that does not seem to be the case, as exposed lobsters did not show any additional damage over lobsters exposed in the other experiments and their exposure to ship noise is periodic, not a singular event. It is also not clear how the level of statocyst damage and any changes in reflex or behaviour might translate in the wild. It has been hypothesised that detection of some component of underwater noise may play a role in migration of lobster pueruli as they transition from their larval pelagic habitat to their post-metamorphosis reefal habitat (Jeffs *et al.* 2005), though the puerulus statocyst does not show the level of development observed in the present study, lacking fluid, hair cells, and secretory pores (Sekiguchi & Terazawa 1997). Furthermore, exposure to aquatic noise has been shown to disrupt foraging behaviour in the shore crab *Carcinus maenas* (Wale *et al.* 2013); reducing common social interactions, aggressive behaviours and tail flips in the crayfish *Procambarus clarkia* (Celi *et al.* 2013); and disrupting communal structure and locomotory patterns, through increases in the frequency, distance and velocity in movements of the lobster *Panulirus elephas* (Filiciotto *et al.* 2014). Despite this, the ecological implications of statocyst damage and slowed righting reflex remain unclear, as the lobsters from the Crayfish Point Reserve in Taroona are thriving (Kordjazi *et al.* 2014), though they are not subject to any fishing pressure and it is plausible that the aquatic noise exposure they are exposed to may also impact the level of predation they face.

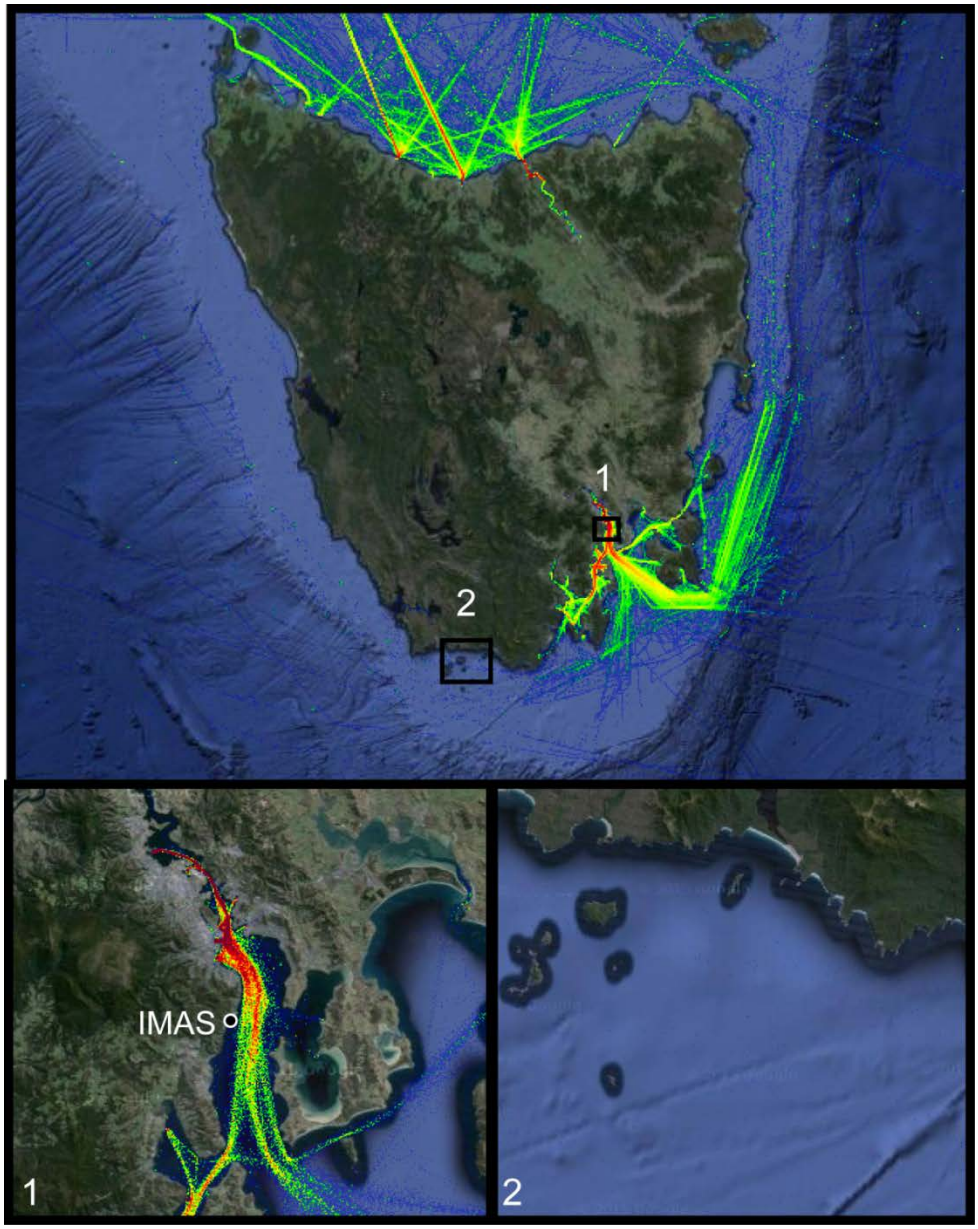


Figure 49. Marine traffic density at lobster collection sites during 2014. Lobsters used in the winter 2014, 150 in^3 standard pressure experiment were collected from site 1, in the Crayfish Point Reserve, Taroona just off IMAS. Lobsters from the winter 2013, 45 in^3 standard pressure, winter 2014, 150 in^3 low pressure and summer 2015, 150 in^3 standard pressure experiments were collected from site 2, around Shoemaker Point off the southern coast. Maps were generated from www.marinetraffic.com.

Haemolymph and condition

Seismic exposure also had a consistent and prolonged negative effect on lobster total haemocyte count (THC) for up to 120 days post-exposure, with decreases in THC ranging from 23% to 60% in the four experiments. These results concur with recent investigations into the response of the European spiny lobster, *Palinurus elephas*, to simulated aquatic noise, with results showing that acoustic stress resulted in reductions in THC ranging from 30% to 70% (Celi *et al.* 2014; Filiciotto *et al.* 2014), though these studies quantified THC on an acute time scale only. THC is commonly used as an assessment of stress and is suggested

to be related to immune competency and health status of crustaceans (Fotedar & Evans 2011). Previous research with spiny lobsters has shown that sick or moribund lobsters have lower THC levels compared to healthy lobsters (Fotedar & Evans 2011). The western rock lobster, *Panulirus cygnus*, showed a 37-55% decline in THC levels in lobsters that were moribund following transportation compared to healthy lobsters, though the stresses of handling and transport were found to increase THC levels by as much as 200% over the short term (Jussila *et al.* 1997).

Given the rapid response of lobster THC to various stressors (Jussila *et al.* 1997; Jussila *et al.* 2001; Celi *et al.* 2014; Filiciotto *et al.* 2014) compared to the chronic response observed in the present study, it is not possible to draw any conclusion as to the mechanism underpinning the observed results. It is important to note, however, that reduced levels of circulating haemocytes have also been reported to be related to nutritional condition of crustaceans (Pascual *et al.* 2006) and therefore the observed response supports the indication of reduced nutritional condition of lobsters as suggested by the Brix index. Interestingly, in the experiment here where THC was measured after 365 days post-exposure, THC levels were elevated more than two fold compared to control lobsters. Dramatic increases in THC of crustaceans has previously been reported in response to bacterial infection (Sequeira *et al.* 1996), raising the possibility that this observed increase in THC of exposed lobster resulted from disease infection following prolonged immune system impairment. However, disease status of lobsters in these experiments were not measured and the lobsters were held in a favourable artificial environment with *ad libitum* access to food and filtered seawater, so further investigation will be necessary to evaluate this hypothesis and what the implications are for lobsters in a more natural environment.

In contrast to the reflex behaviour and haemocytic impacts, seismic exposure only significantly influenced two of the 25 humoral haemolymph parameters investigated, that of the Brix index in the winter 2014, 150 in^3 low pressure experiment and THC in all 4 experiments. Brix index is a measure of the refractive index of a fluid which has long been used in veterinary and medical laboratories as a rapid method to determine solute concentration in bodily fluids (George 2001). More recently, Brix index has been shown to correlate closely with haemolymph nutrients such as proteins, triglyceride and cholesterol, and whole body condition factors including abdominal muscle and hepatopancreas gross energy as well as hepatopancreas total lipid content of lobsters (Oliver & MacDiarmid 2001; Simon *et al.* 2015). Brix index is therefore considered a reliable indicator of nutritional condition of lobsters with a reduced Brix representing poorer nutritional condition. The finding of reduced Brix index of exposed lobsters in the winter 2014, 150 in^3 low pressure experiment suggests a chronic impairment of nutritional capacity resulting in diminished nutritional condition of lobsters after 120 days post-exposure. This is in contrast to research with snow crab (*Chionoecetes opilio*) where seismic exposure had no influence on haemolymph refractive index (Christian *et al.* 2003). However, this previous study only examined refractive index of crabs immediately after exposure and thus would not reveal chronic effects on nutritional capacity. In the present study, the indication of nutritional impairment as indicated by haemolymph refractive index, was not consistently supported by

other whole body measures of nutritional condition, including whole body wet weight and hepatopancreas index, however both these measures have been shown to have limited value for predicting nutritional status of feeding lobsters, with refractive index offering a superior indication (Cockcroft 1997; Simon *et al.* 2015). Reduced nutritional capacity could be caused by numerous factors, including reduced feeding ability, impaired digestive or assimilation capacity and diminished competitive advantage within the social hierarchy. However, as none of these factors were recorded in our experiments it is impossible to infer the mechanism for reduced nutritional capacity.

Whilst the study found some evidence of an effect of seismic exposure on lobster nutritional capacity in winter 2014, 150 in^3 low pressure experiment (i.e. reduced Brix index), this result was not repeated in the winter 2014, 150 in^3 standard pressure experiment where a greater impact may be expected due to the higher energy of the acoustic signal. Currently it is unclear why this effect on Brix index was not consistent between experiments, however, it is possibly related to population differences in lobsters used in the different experiments, as previously discussed in relation to statocyst damage.

Overall nutritional condition of both exposed and control lobsters improved considerably in the 120 day post-exposure experiment period in both the winter 2014, 150 in^3 low pressure and winter 2014, 150 in^3 standard pressure experiments. Improved nutritional condition of lobsters was evident by the significant influence of time on several haemolymph parameters, including increases in Brix index, calcium, phosphorus, total protein, triglyceride, cholesterol, and glucose concentration, all of which have been shown to relate to nutritional condition of lobsters (Simon *et al.* 2015). Improved nutritional condition of lobsters during this period would be expected due to the development through the moult cycle and unlimited feed availability in captivity. Female lobsters used in the present study were early post moult at collection, when nutritional condition would be poorest due to energy demanding physiological processes and a period of fasting associated with the moult (Simon *et al.* 2015). Feeding during the intermoult period restores depleted energy reserves and supports tissue growth (Musgrove 2001; Simon *et al.* 2015).

In conclusion, these results indicated that seismic exposure has little impact on electrolyte, metabolite and enzyme balance which suggests that the haematological homeostasis of *J. edwardsii* is reasonably resilient to seismic acoustic signals. However, refractive (Brix) index of lobster haemolymph declined after 120 post-exposure in one experiment and lobsters in all experiments had a sustained modification of THC suggesting potential negative influence of seismic exposure on lobster nutritional and immunological capacity. The biological or ecological significance of this physiological impairment is difficult to gage. There was no effect of seismic exposure of lobster survival and nutritional condition of both control and exposed lobsters improved considerably during the prolonged period post-exposure period (120-365 days) which suggests that the physiological impairment associated to seismic exposure was relatively minor. However, this assessment was based on lobsters maintained in controlled conditions in captivity with limitless supplies of highly nutritious feed. Lobsters in the wild would likely be subjected to more stressful conditions associated with limited access to lower energy feed, predator risk and disease exposure. It is likely that minor physiological

impairment would have a much greater consequence for animal fitness in these more difficult wild conditions. Further research on the effects of seismic exposure on the performance of lobsters in the wild is required to better understand the biological/ecological consequence of seismic exploration for this important group of crustaceans.

Larval development, quantity and quality

To assess the impact of air gun exposure on embryo development, three primary concerns were investigated. The first was the loss of eggs either through direct mortality or as a result of over-grooming of the egg bundle by the female, which is a known behavioural response to stress (Smith & Ritar 2005). This concern was not supported, as exposure to signals from seismic air guns did not result in any apparent egg bundle loss, nor were there any differences in fecundity between control and exposed lobsters from any of the three exposure levels. The fecundity of the lobsters used in this study was on par with that of previous reports for similar sized *J. edwardsii* (Annala & Bycroft 1987; Tong *et al.* 2000). It was observed that both the control and exposed treatments in the winter 2014, 150 in^3 low pressure experiment hatched considerably less larvae than in the other two experiments, though this was not examined statistically. Given the lack of difference between control and exposed treatments in this experiment, along with the fact that lobsters for this experiment were collected from the same site as the winter 2013, 45 in^3 standard pressure experiment and were approximately the same age (based on carapace length), this apparently low fecundity relative to that of lobsters exposed to a lower SEL in the 2013 experiment and a higher SEL in the winter 2014, 150 in^3 standard pressure experiment cannot be attributed to air gun exposure. Based on the consistency in the collection, transportation and animal husbandry methods between experiments and the consideration that the females were berried prior to collection for the experiment, the most parsimonious explanation for this result is natural variation in clutch size.

The second primary concern regarded the quality of the larvae, with *a priori* expectations that exposure may result in reduced larval energy content or larval competency, as assessed using a well-established activity test developed specifically for *J. edwardsii* larvae that correlates activity in a reduced salinity, increased temperature environment with the rate of survival through phyllosoma moulting stages (Smith *et al.* 2003b). Again, this concern was not supported, as no difference was found in either larval energy or competency at any of the three levels of exposure.

The third concern, that exposure would result in abnormal larval morphology, cannot be immediately dismissed. Although no apparent morphological abnormalities were observed, exposed larvae from the winter 2013, 45 in^3 standard pressure experiment were found to be significantly longer than control larvae. Larval length in crustaceans shows a substantial degree of natural variability and can be affected by a range of factors (Fox & Czesak 2000; Jacobs & Podolsky 2010), including biotic influences such as maternal size and maturity (Ouellet & Plante 2004; Moland *et al.* 2010) and abiotic factors such as differences in temperature (Tong *et al.* 2000; Smith *et al.* 2003a; Bermudes & Ritar 2008) and photoperiod (Bermudes & Ritar 2008; Smith *et al.* 2003a). Indeed, the size of larvae in this study fell well

within the range for Stage I larval length of *J. edwardsii* reported by Lesser (1978), indicating that the range of natural variation in larvae is much greater that the differences observed between treatments in this study. Furthermore, these morphological differences were not found to translate to any difference in either larval energy content or competency despite the expectation that larger larvae should be more competent than smaller larvae (Tong *et al.* 2000). Whether or not the observed differences in size are biologically significant, seismic exposure did not result in a decrease in fecundity, either through a reduction in the average number of hatched larvae or as a result of high larval mortality; compromised larvae or morphological abnormalities, thus none of the three concerns over embryonic exposure to seismic air gun signals were supported. These results support the suggestion that early life stage crustaceans may be more resilient to seismic air gun exposure than other marine organisms (Pearson *et al.* 1994).

Indeed, the evidence suggesting seismic exposure negatively affects the embryos of marine invertebrates is limited and questions must be raised regarding the methods of these studies. A recent study of New Zealand scallops (*P. novazelandiae*) exposed to recordings of an air gun played using an acoustic projector in a tank found larvae hatched following embryonic sound exposure suffered significantly delayed development and a nearly 50% occurrence of growth abnormality (Aguilar de Soto *et al.* 2013). Based on these results, the authors raised concerns about the impacts of seismic exploration in spawning areas of marine invertebrates. However, the results from acoustic work in tanks cannot be put into real world context, as the long wavelengths produced by real sources such as an air gun cannot be emulated in a small tank. First, real sources cannot be used in tanks, creating a problem in emulating the physics of the source. Second, sound bounces off tank surfaces, resulting in large amounts of constructive or destructive interference at small spatial scales, as well as the creation of a complex and unpredictable relationship between sound pressure and particle motion (Popper & Fay 1993). Similarly, experiments have been performed in extremely shallow water depths (e.g. Payne *et al.* 2007; Pearson *et al.* 1994) which risks overestimation of the level of acoustic energy experimental animals receive as phase cancellation creates a “sound shadow” resultant from sound waves reflecting from the water’s surface (Urick 1983; McCauley *et al.* 2003). Finally, methods must be either biologically relevant or experimentally validated if results are to be extrapolated to real world conditions. Seismic exposure was suggested to result in significantly higher rates of mortality and significantly delayed development in snow crab (*C. opilio*) embryos (Christian *et al.* 2003), however, this experiment was performed on eggs stripped from the females and cultured in a laboratory for six weeks prior to exposure and eighteen weeks following exposure. Subsequent work on larvae that had been exposed to air gun signals as embryos but were allowed to hatch normally without being stripped from berried females did not suffer any negative effects (Payne *et al.* 2008b). In light of the emerging trend in which the deleterious results observed in laboratory studies are not supported by the results of field based experiments, it is apparent that results from the field are necessary before laboratory studies can be relied upon to supplement our understanding of effects in the field and inform any meaningful conclusions of seismic air gun exposure.

It must be noted that, at the time of exposure in the present study, the spiny lobster eggs were at an early embryonic developmental stage, just after extrusion and prior to eye development, and were thus entirely soft tissue with no large internal density differences. Such large internal density differences could cause localised transfer of high intensity acoustic energy to physical forces within the egg. Later spiny lobster larval developmental stages have developed sensory systems including arrays of pinnate setae along the flagella of the antennae and mechanosensory statocyst organs which they may use for navigation during the critical onshore migration and settlement phase (i.e. Jeffs *et al.* 2005; Fitzgibbon *et al.* 2014). As such, the experimental results found here may not necessarily be the same for spiny lobsters exposed later in development (including later stage embryos and larvae) and is an area which requires further research to determine the potential impacts of seismic surveys on lobster populations. Until such information is available, an inability to draw conclusions on the effects of air gun exposure will persist, preventing the development of evidence-based regulation for seismic surveys.

Scallops

The purpose of the present study was to evaluate the effects of exposure to seismic air gun signals on scallops, to determine whether 1) exposure resulted in immediate mass mortality, 2) whether exposure substantially affected behaviour and 3) whether any changes to behaviour resulted in mortality in the longer term. To that end, scallops were exposed to signals from a single air gun with 45 in^3 or 150 in^3 chambers at comparatively short range (km to < 10 m closest approach) to emulate exposure from a larger commercial seismic source. In addition, these experiments utilised an exposure regime consisting of multiple pass-bys to emulate the repeated exposure benthic animals such as scallops might receive during exposure to a real-world commercial seismic survey, given the comprehensive coverage of survey transects and the efficiency of seismic signals in the water.

Mortality

For the present study, mass mortality was considered “an unusual and sharply defined increase in mortality rate of sufficient proportion to affect population size significantly” (Sindermann 1996). Based on the results of three experiments exposing the scallop *Pecten fumatus* to 4 different treatment levels using two different sizes of air gun with sampling conducted over a 4 month period, the hypothesis that exposure causes immediate mass mortality may be rejected. The only case of mass mortality observed in the course of this study occurred in the 2015 summer experiment, when both the control and exposed treatments scheduled for sampling on day 120 suffered complete mortality. Indeed, when compared to naturally occurring annual mortality rates of 11-51% and a 6-year mean mortality rate of 38% in *Pecten fumatus* (Coleman & Gwyther 1988), the experimental mortality rates at 120 days post seismic air gun exposure, at between 9.4% and 20%, fall towards the low end of what might be expected. Even the highest levels of mortality recorded in this experiment, the 17.5% and 20% suffered by 4-pass treatments from the 2014 and 2015 experiments, were modest compared to naturally occurring mortality rates.

Although there was no support for the mass mortality hypothesis, the results indicate that exposure, particularly repeated exposure, resulted in significantly increased mortality compared to unexposed controls, with 2- and 4-pass treatments found to have an elevated risk of daily mortality over that of both 0- and 1-pass treatments. Rather than being immediate, the majority of mortality occurred over the longer term, with approximately 60% of deaths in both the 2013 and 2014 experiments observed at the day 120 sample point. In light of the well-established link between fishery stress and mass mortality in scallops (Medcof & Bourne 1964; Coleman & Gwyther 1988; Naidu 1988), the significant trend between air gun exposure and medium- to long-term mortality in this study raises concern that, although mass mortality did not occur within the 120 day scope of these experiments, stress resulting from seismic exposure may undermine the health of exposed scallops and serve as an ultimate, or distal, cause to a mass mortality event at a later time in response to an unrelated stressor. As such, a better understanding of the sub-lethal effects of seismic exposure is necessary for a more complete understanding of the effects of seismic exposure on scallops.

Haemolymph

Exposure to seismic air gun signals also had a considerable effect on the physiology of *P. fumatus*, as indicated by differences in the haemocytic and humoral components of haemolymph in the three experiments that comprised this study. The number of circulating haemocytes demonstrated dose- and time-dependent responses and chronic disruption lasting the entirety of the 120 day experiments. Similarly, in the humoral component of haemolymph, all mineral and electrolyte ions assayed showed a significant response to exposure, which was again evident over the long term.

In putting these results into context, there are three considerations to keep in mind regarding the results reported here and the use of haemolymph analysis to quantify and interpret the physiological status of the scallops and bivalves in general. First, the haemolymph results reported here were collected only from living scallops, and as such, there is a distinct possibility of underestimation of the degree of severity exposure had, as any imbalances extreme enough to result in mortality have not been detected. Second, due to the open circulatory system in scallops and other bivalves, haemolymph parameters can vary considerably between individuals (Fisher *et al.* 1996; Ford & Paillard 2007; Flye-Sainte-Marie *et al.* 2009), potentially introducing noise into the data and obscuring some of the impacts. With this in mind, comparisons of haemolymph results between treatment levels from this study and to reports in the literature of other bivalves and molluscs focus primarily on observed trends rather than specific values. Finally, considering the depth and breadth of alterations in haemolymph parameters resulting from exposure, it is not possible to positively identify the actual impact air gun exposure had on scallops. Instead, the findings are compared to evidence from other stressors to suggest potential impacts and approaches for subsequent research into the proximate causes for the observed results.

The first trend to consider is that of circulating haemocytes in bivalves, which has been well characterised in response to biotic (i.e. tissue damage; bacterial, parasitic and viral infection) and abiotic (i.e. oxidative stress from emersion, exposure to heavy metals or polycyclic

aromatic hydrocarbons) stressors. The typical pattern of fluctuations in haemocyte numbers (Cheng 1987; Suresh & Mohandas 1990; Anderston *et al.* 1995; Livingstone *et al.* 2000; Hannem *et al.* 2009; Hannem *et al.* 2010a) begins with a rapid (*ca.* ≤24 h) decline in THC as circulating haemocytes are mobilised to areas of damage (Cheng 1987; Suresh & Mohandas 1990; Hannem *et al.* 2010b). A subsequent increase follows, with THC either returning to (Hannam *et al.* 2010a) or exceeding (Jones *et al.* 1993) baseline levels, resulting from stimulation of haematopoesis and may last from several days to several weeks depending on the nature of the stressor (Anderson *et al.* 1995; Pipe *et al.* 1999; Hannem *et al.* 2009; Hannem *et al.* 2010a), before THC returns to baseline levels over the long term. Indeed, this trend is important for the characterisation of haematological response, as comparisons of THC values between studies are difficult due to differences in where haemolymph is collected (i.e. adductor sinus, pericardial sinus, extrapallial fluid) counting methods (i.e. flow cytometers, haemocytometers or the preparation of stained slides), as well as natural variation, with factors such as seasonal fluctuations (Delaporte *et al.* 2006; Flye-Sainte-Marie *et al.* 2009; Lin *et al.* 2012), nutritional status (Anderson *et al.* 1995; Delaporte *et al.* 2003; Delaporte *et al.* 2007), changes associated with reproductive state (Delaporte *et al.* 2006; Flye-Sainte-Marie *et al.* 2009; Lin *et al.* 2012), an inverse relationship with size (Suresh & Mohandas 1990; Flye-Sainte-Marie *et al.* 2009; Lin *et al.* 2012) and changes in haemolymph volume due to the open nature of the bivalve circulatory system.

Seismic exposure in each of the experiments did not elicit this exact trend, though each experiment showed some similarities to the typical response. Scallops in the 2- and 4-pass treatments demonstrated the predicted initial rapid decline in THC relative to 0-pass controls at day 0 in the 2013 experiment, but not in the 2014 experiment. At day 14, 0-pass scallops from the 2013 experiment showed an unexpected reduction in circulating haemocytes to levels similar to that of exposed scallops, whereas in both the 2014 and 2015 experiments, exposed scallops demonstrated the expected over-compensatory response, with scallops receiving the highest level of exposure showing significantly elevated THC, relative to both the previous sampling point (2014) and to control scallops (both 2014 and 2015).

The observed difference between treatments at day 0 and the lack of difference at day 14 in the 2013 scallops may be indicative of a latent response to the stresses resulting from dredging and repeated transportation (Maguire 2002a; 2002b) further exacerbated by seismic exposure. Along the same lines, the lack of an initial decrease in THC of exposed scallops in the 2014 experiment may reflect a comparatively gentle treatment resulting from hand-collection. Furthermore, the 2014 day 0 results suggest that scallops were probably sampled too soon following exposure, as the <5 h between exposure and the completion of sampling may not have allowed enough time to demonstrate a response absent the effects of non-seismic stressors incurred in the 2013 experiment. The similarity in THC levels in the 0-pass control treatments in the 2013 and 2014 experiments may be an indication that the 2013 scallops had returned to baseline levels by the end of the experiment. Finally, in both experiments, all exposed treatments were found to have significantly lower THC levels than controls, indicating that exposure, even at low levels (i.e. 1-pass compared to 4-passes), resulted in a substantial reduction of the number of circulating haemocytes over a chronic

time scale. Similar disruption of haemocyte levels have been explained as a down-regulation of haematopoesis resulting from the unsustainably high energetically demands incurred by long-term elevation of haemocyte production (Cheng 1981; Cheng & Sullivan 1984; Pipe *et al.* 1999). Additionally, the scallops exposed to the more intense signals from the 150 in^3 air gun in the 2014 experiment had substantially lower THC levels than scallops from the 2013 experiment exposed to the 45 in^3 air gun potentially indicating an intensity dependent dose response in the long term rather than the response to the cumulative number of exposures observed at day 14 of the 2014 experiment.

Extrapolation of this response to understand the impacts on scallop populations following exposure to a seismic survey remains difficult for a number of reasons. First, data on haemocyte counts and the general response to stressors in pectinid scallops are scarce, and a better understanding of the natural, temporal, developmental and spatial variation in scallop taxa would enhance assessment of impacts. Second, a better resolved characterisation of THC levels over the short- and intermediate-term following exposure is required to determine whether seismic exposure elicits the typical haematological response of better understood stressors, as differences in the collection methods of scallops used in this study prevented conclusive determination. Third, the sampling regime used in this study could be improved upon with more frequent sampling between days 0 and 14 to better resolve the temporal aspect of haemocyte response. Once these basic aspects of scallop haematology have been understood, more sophisticated investigations using direct measurements of immune function (i.e. differential haemocyte counts; assays of phagocytosis, membrane stability, etc.) will provide greater insight into the physiological effects of exposure, as even if haemocyte numbers return to normal or higher than normal, their function may be compromised (Hannem *et al.* 2009; Hannem *et al.* 2010a).

In comparison to the haemocytic response to exposure, the humoral response is more difficult to put into context with previous reports of responses to stressors in bivalves. Haemolymph pH showed considerable variation between experiments, namely through very high pH levels across all treatment levels at days 0 and 14 in the 2013 experiment compared to the 2014 and 2015 experiments. This difference may be similar or linked to the THC results in that seismic exposure exacerbated the stress resulting from dredging during collection and transport, however, further investigation into any synergistic effects are necessary to make such a suggestion.

The causes underlying elevated pH in exposed scallops are not well understood at this point, as reports of alkalosis in bivalves are rare in the literature, with only one report in a bivalve, the pacific oyster (*Crassostrea gigas*), in which handling, shell drilling, cannulation and repeated drawing of haemolymph was attributed to significant alkalosis that progressed over an 8 h period, before returning to baseline levels by 12 h (Jones *et al.* 1993). Alkalosis has also been reported to occur in the coelomic fluid of the peanut worm (*Sipunculus nudus*), in which hypoxia induced a 12 h period of elevated pH accompanied by a decrease in bicarbonate concentration (Portner *et al.* 1984), which was attributed to the consumption of protons during the breakdown of phospho-L-arginine during early anaerobiosis and the return to baseline levels and subsequent acidosis reflected the metabolic shift from the Embden-

Meyerhof-Parnas pathway to the succinate-propionate pathway of anaerobic metabolism. Hypoxia, both environmental and functional, has been reported to induce alkalosis in a range of cephalopods (Houlihan *et al.* 1982; Portner *et al.* 1987; Portner *et al.* 1990; Portner *et al.* 1991; Siebel *et al.* 2014), The degree of alkalosis varied in scope from less than 0.1 to as much as 0.5 pH units (Portner *et al.* 1991; Siebel *et al.* 2014) and lasted for a short period of time (minutes to hours) during the return to steady-state conditions and the recovery from hypoxia. The rise in pH has been attributed to two factors: increased proton exchange during haemocyanin deoxygenation (Houlihan *et al.* 1982; Portner *et al.* 1991), and the mobilisation of bicarbonate from intracellular spaces to haemolymph (Portner *et al.* 1991), rather than hyperventiliation, which was dismissed as a cause due to the coupling of ventilation and locomotion in the cephalopods investigated along with previous observation of reduced ventilation rates under hypoxic conditions (Siebel *et al.* 2014).

There are considerable differences between the reported cases of alkalosis in cephalopods and its occurrence in scallops in the present study. In all cases, alkalosis in cephalopods was in response to hypoxic conditions and was accompanied by increases in haemolymph bicarbonate concentration. In comparison, scallops were in natural seawater prior to the day 0 measurements, in tanks supplied with highly aerated natural seawater prior to day 14 measurements and in natural seawater prior to the day 120 measurements. The occurrence of hypoxia and anaerobiosis in aerated water could be explained if air gun signals elicited metabolically expensive behaviour in scallops, such as extended periods of valve closure or swimming, however, this hypothesis was ruled out based on video recordings during exposure. Furthermore, haemolymph bicarbonate concentration decreased in scallops, showing a strong and statistically significant dose-dependent negative response to the level of exposure, a result contradictory to the results from cephalopod experiments. Finally, scallop alkalosis was persistent for at least 14 days, far longer than the scale of hours previously reported in any invertebrate. At this stage, given the substantially different circumstances between alkalosis in scallops and cephalopods, it seems probable that different mechanistic factors are at play, though the mechanisms responsible in scallops remain unclear.

At this stage, it is similarly difficult to precisely ascribe the disruption of osmo/ionoregulation to air gun exposure. Owing to adaptation to the stable nature of their sub-littoral habitat, scallops show a limited capacity for regulation of haemolymph ion concentration (Wada 1984; Christophersen & Strand 2003), and tend to show concentrations similar to that of the surrounding seawater with some limited exceptions such as potassium and bicarbonate, which may be slightly elevated relative to seawater (Shumway 1977; Burton 1983) and some evidence suggests that glucose and protein levels may be regulated (Ford & Paillard 2007), yet a broad scope of changes was observed, with every mineral and electrolyte assayed showing a significant alteration. The fact that some ions (i.e. Na, K, Cl, Ca) increased in concentration while others (i.e. Mg, bicarbonate) decreased further confuses the issue. In addition, both protein and glucose levels showed a long term decreasing trend in response to high levels of exposure.

Although it is only possible to speculate at this point, if seismic exposure resulted in tissue damage in scallops, some of the alterations in haemolymph ionic parameters could be

explained by such damage. One possibility is that the gills suffered damage, either due to the waterborne pressure wave/particle motion or the ground borne vibration produced by the air gun signal violently shaking the animal. If such damage occurred, it would explain the observed haemocyte response, as circulating haemocytes would amass at the site of damage and haematopoesis would subsequently be stimulated and the resulting collapse in THC may reflect the inability of immature haematocytes to adequately deal with the impacts of the damage over the long term. Gill tissue damage would also result in a reduction in the capacity for oxygen transfer, resulting in hypoxia and subsequent alkalosis. The increase in pH and the corresponding increase in calcium concentration would contribute to enhanced haemocyanin oxygen affinity (Burton 1983), potentially ameliorating reduced oxygen transfer in the damaged gill tissue, however, there is no evidence to suggest that scallops have such a capacity for regulation.

A second hypothesis is the incurrence of tissue damage to components of the excretory system, such as the kidney or auricular pericardial glands, which function in the filtration, storage and secretion of metal ions from the haemolymph (Andrews 1988; Haszprunar 1996). In both sites, metal ions form concretions called nephroliths, either for the purpose of storage or detoxification, which may be excreted as solids, re-absorbed into haemolymph and excreted as solutes (Franc 1960 (referenced in Beninger & Le Pennec 2006); Simkiss 1976; Coombs & George 1977). Identification of the metals sequestered in the nephroliths show that they contain what appears to be species-specific concentrations of a range of metals (including Ca, P, Mg, K and Cl) removed from the urine and haemolymph (Carmichael *et al.* 1979; George *et al.* 1980). Perhaps the pressure wave or vibratory ground motion from the air gun signals cause these nephroliths to act as a transducer as suggested above in regards to the statoliths of the statocyst, resulting in damage to the cellular structure of the excretory system, causing the results observed in the haemocytes and reducing the capacity for haemolymph filtration and metal ion regulation.

Behaviour

A supposition of the mass mortality hypothesis was that air gun exposure would alter normal scallop behaviour, through either extended periods of swimming or valve closure, either of which would lead to a depletion of energy stores and, ultimately, death (Harrington *et al.* 2010). In the 2014 and 2015 experiments, only 4 instances of swimming were observed, thus this portion of the hypothesis cannot be supported. In regards to extended valve closure, which has been previously documented in response to decreased salinity (Duggan 1975; Strand *et al.* 1993) and fluctuations in temperature (Strand *et al.* 1993; Jonasson *et al.* 2004), only 2 individual scallops were observed to remain closed for extended periods, both of which remained closed for the entire duration of recording. To further test this hypothesis, tentacle retraction was used as a proxy for valve closure and the proportion of time scallops spent with tentacles extended, partially extended or retracted were compared. Exposure did not elicit any marked change in tentacle behaviour, with scallops receiving the highest levels of exposure demonstrating proportions of tentacle extension on par with that of unexposed scallops. In all treatments from 2014 and 2015 experiments, scallops spent the majority of time with tentacles fully extended and retraction made up a very small component of

behaviour. It must be noted, however, that in the 2015 experiment, exposed scallops displayed relatively low levels of tentacle extension and high levels of tentacle retraction compared to exposed scallops from the 2014 experiment. Although this trend was not significant, the possibility that limited sample size contributed to this result cannot be ignored and further investigation into the effects of exposure during warm summer conditions is warranted.

It is clear, however, that seismic air gun exposure altered scallop behaviour, with normal behavioural patterns (i.e. responses to visual cues; valve closures; tentacle retractions; "coughs" used to irrigate the mantle cavity; and locomotory behaviours, such as swimming or repositioning) disrupted in two ways. First, exposed scallops demonstrated a marked reduction in these classic behaviours. Second, exposed scallops exhibited a novel velar flinch behaviour. This novel flinching behaviour was not observed in 0-pass control scallops, and in exposed scallops, was observed exclusively during the intra-exposure period, generally in direct response to an air gun signal at close range. It occurred in 50% of 1-pass scallops, 100% of 2-pass scallops and 75% of 4-pass scallops from the 2014 experiment and in 85% of 4-pass scallops in the 2015 experiment, with the frequency of occurrence in 2-pass and both 4-pass treatments significantly greater than predicted based on the time of observation. It is not clear whether the lower incidence of this behaviour in 1-pass scallops relative to the higher exposure treatments was a function of cumulative exposure, or whether it was a matter of opportunity, as the 1-pass air gun run in 2014 was substantially longer in its lead up run than any of the other runs, thus any individual scallop had a lower proportion of time when the air gun was in close range than in other treatments (since exposures involved a limited number of air gun signals). Considering the flinch behaviour appeared to occur in response to close range signals and slightly before the waterborne arrival (as indicated by recorded sound on the video), it is probable that the behaviour occurs only for high level signals and possibly in response to ground borne energy, which arrives slightly before waterborne energy as the compressional speed of sound is higher in the substrate than in the water. If that is the case, the flinching behaviour may not be replicated in circumstances where the air gun is a greater distance from the scallop, such as if the seismic vessel is operating in deeper water, although many of the most heavily fishery-exploited scallop species commonly occur at depths less than 50 metres (Brand 2006).

Reflex behaviours

In addition to the analysis of behaviour during seismic exposure, the righting and recessing reflexes of scallops, which are affected similarly using jets of water directed with precise control by the mantle to accurately position the scallop, were compared between exposure levels. Scallops use righting to re-orient themselves when overturned, and the reflex has been suggested to be a rapid and objective measure of vitality, as factors such as desiccation (Minchin *et al.* 2000a) and dredging stress (Maguire *et al.* 1999; Maguire *et al.* 2002) have been shown to cause delays in righting. Once scallops right themselves they tend to recess into the sediment, a reflex first described by Baird (1958), who observed that scallops use jets of water to form a depression in the sand and then use a powerful jet to propel themselves off the seabed and land in the depression with extraordinary precision and control. Recessing has

since been understood to confer a number of benefits, such as assisting feeding, protection from predators, prevention of shell fouling and reduction of the scallop's hydrodynamic profile in high currents (Maguire *et al.* 1999). Indeed, it has been noted that being recessed in the sediment appears to be the "natural" state for scallops, which is supported by both observations in the wild and the laboratory (Baird 1958; Minchin 2003).

In this study, righting was compared only for the 2015 summer experiment. This limited investigation showed results consistent with previous investigations of the effects of desiccation and dredging stress on the reflex, as exposed 4-pass scallops had a mean righting time nearly 40% longer than 0-pass control scallops. It is important to note that this test was conducted 14 days post-exposure, indicating that the disruption in normal reflex function has the potential to be a persistent issue. Interestingly, although it has been suggested that being upside down may cause stress in scallops (Minchin *et al.* 2000a), differences in righting time are not necessarily associated with compromised physiology or behavioural competency, as cultured scallops were found to right themselves at the same rate as wild scallops despite having a higher glycogen content and a more vigorous escape response (Lafrance *et al.* 2003). Based upon the lack of energetically expensive behaviours observed in response to air gun signals, it can be assumed that the reduced capacity for righting in 4-pass scallops is not likely a result of any energetic or biochemical difference.

Unlike the righting results, scallops in this study exposed to air gun signals recessed more rapidly than control scallops. This was a surprising result, as previous studies of recessing time have showed that stressors such as desiccation (Maguire *et al.* 1999; Minchin *et al.* 2000a) and simulated dredging (Maguire *et al.* 2002a, 2002b) result in slower recessing. Although comparisons of mean recessing times between different studies are problematic due to the size-, seasonal- and substrate-dependent (Fleury *et al.* 1997; Maguire *et al.* 1999) nature of recessing times, a clear trend towards increased levels of stress resulting in increased recessing time exists in the literature. Such increases have been explained as a result of energy depletion during exposure to stressors, so the lack of any swimming or closing behaviour during air gun exposure may put the unexpected recessing response in this study in context, though the more rapid recessing of exposed scallops compared to control scallops cannot easily be explained.

Recessing time for 0-pass scallops varied between 48 and 72 h, with control groups from both the 2013 experiment and the 2015 experiments having a mean recessing time of 60 h. Both of these experiments saw scallops under additional stress, with the 2013 experiment conducted on dredged scallops and the 2015 experiment performed in summer. Along similar lines, the second 2014 recessing test was performed 4 months post-exposure, when scallops had entered into a different reproductive state. Conversely, exposed scallops, regardless of exposure level, did not display a great deal of variation in recessing time. The 2013 experiment saw exposed scallops recesses with a response that was inversely proportional to exposure, but in the other 2 experiments, exposed scallops recessed at similar rates. Perhaps the most important finding from these recessing tests is that the impact persisted to the 120 day sampling point in the 2014 experiment, indicating a chronic alteration in this reflex. This stands in stark contrast to previous investigations into the effects of stressors on recessing, as

daily simulated dredging disturbances did not result in a cumulative effect, indicating scallops had recovered within 1-3 days (Fleury *et al.* 1996; Maguire *et al.* 1999; Maguire *et al.* 2002b).

At this point, it is unclear why seismic exposure would result in a recessing response opposite to that of other stressors while the righting reflex corresponds with previous results. Perhaps the effect of air gun exposure is less of a physiological stress, when compared to the oxidative stress of emersion or the energy depletion caused by dredging, and more of a problem with the sensory system of the scallop. The primary mechanosensory organs in scallops are the statocysts, a paired (though asymmetrically sized) sensory organ located near the pedal ganglion consisting of a spherical sac that is lined with hair cells that provide a sense of balance as a gravity receptor via contact between a statolith (which may be either a single statolith or multiple statoconia cemented together) and the sensory hair cells (Buddelmann 1988). In addition to the statocysts, scallops may have a second organ that plays a role in mechanoreception: the abdominal sense organ, a sickle-shaped pocket in the mantle fold, adjacent to the anus and the adductor that is densely populated with sensory hair cells (Zhadan *et al.* 2004; Beninger and Le Pennec 2006). Although the abdominal sense organ has been suggested to perform several roles, such as chemoreception and the regulation of feeding via the monitoring of water flow through the mantle (Charles 1966; Moir 1977), there is compelling evidence that it functions in mechanoreception, detecting both water- and ground-borne vibrations as well as providing directional sensitivity (Zhadan & Semen'kov 1984; Haszprunar 1985; Zhadan *et al.* 2005).

For both organs, there is potential for the high energy impulse from air gun signals to cause mechanical damage to the hair cells responsible for sensory detection, particularly in the case of the statocyst, which relies on the communication between the statolith with hair cells to transduce vibrational force into electrical impulse. In the case of the statocyst, damage to the hair cells would appear to explain the compromised righting reflex, though it must be noted that Buddenbrock (1915) reported that statocyst ablation did not impair the righting reflex in nine species of pectinid scallop. There is some evidence that acoustic signals can damage the statocysts of other marine organisms, as exposure to low frequency signals in a laboratory environment resulted in severe, progressive damage in a range of cephalopod species (André *et al.* 2011; Solé *et al.* 2013).

Although the mechanoreceptor capacity of the abdominal sense organ is not well understood, it shows a high level of sensitivity to vibrations in the water from 20-1500 Hz (Zhadan & Semen'kov 1984), a range which encompasses the approximately 20-200 Hz dominant frequency typical of seismic air gun signals (McCauley *et al.* 2003; Tashmukhambetov *et al.* 2008). Damage to this organ may explain the unexpected recessing results, as increasing levels of exposure resulting in increasingly rapid recessing may indicate a progressive level of abdominal sense organ disruption and a corresponding progressive decrease in mechanoreception and directional sensitivity. If the abnormal reflex results found in this study are indicative of damage to mechanosensory organs, exposed scallops may face considerable ecological ramifications. For example, in experiments on 9 species of pectinid scallops, disruption of the statocyst nerve resulted in the loss of the ability to control the

vertical component of swimming (Buddenbrock 1915), compromising a primary aspect of predator avoidance. The abdominal sense organ has also been suggested to contribute to predator detection, with the detection of water-borne vibrations originating from above the scallop filling in a blind spot of the visual, tactile and chemoreceptive systems (Zhadan 2005). Both organs warrant additional investigation, with histological and electrophysiological approaches likely avenues for improving our current understanding of what roles these organs play and how they may be affected by air gun exposure.

Condition

To evaluate whether any sub-lethal effects impacted scallops in ways relevant to their value to fisheries, five indices were compared between treatment levels. These indices were chosen from previously published literature based on their linkage of easy to acquire measurements (e.g. adductor mass, shell mass, etc.) to physiological responses to pollution stress (Hannem *et al.* 2010a) and differences in culturing practices (Kleinman *et al.* 1996; Maguire *et al.* 1999).

Neither mass-to-length nor mass-to-volume ratios showed any response to exposure across the 3 sample points. The former had previously shown a rapid response in *Chlamys islandica* after 2 days of exposure to oil contamination, which the authors attributed to glycogen store depletion resultant from the metabolism of polycyclic aromatic hydrocarbons (Hannem *et al.* 2010a). Similarly, mass-to-volume ratio, which had previously been used to compare scallop spat raised at different stocking densities with differences in the index found to be proportional to differences in glycogen content (Maguire *et al.* 1999), was consistent throughout the study with no differences between treatments or at sample times.

The third index used in the present study was tissue mass relative to total mass, which showed significant differences for both the 2013 and 2014 experiments, with the 2013 experiment showing a marked reduction at the 120 day sampling point compared to the previous two sampling points and the 2014 experiment following this trend to a lesser degree. In both experiments, this decrease was driven largely by shell fouling, as scallops at the day 120 sampling point accumulated both algae and sessile animals on their shells while suspended from mussel leases.

The final two indices relate adductor mass to total mass and tissue mass, respectively, making these indices adapted from Kleinman *et al.* (1996) of particular concern for the fishery value of scallops. Neither 2013 nor 2014 experiments showed any significant differences for these indices, although 2013 scallops had a marked increase in both at the day 120 sample point, which again was driven by the reduction of gonad tissue following spawning. In the 2015 experiment, however, both indices showed that 0-pass control scallops had significantly greater adductor mass relative to total mass and tissue mass. The cause of this difference is not clear, as video analysis of scallops did not show any difference in energetically costly behaviours such as swimming in exposed scallops. Temperature likely played a role, with air gun exposure potentially acting as a synergistic stressor in combination with austral summer water temperatures. This finding has potentially important ramifications for the scallop

industry, as there may be greater risk of seismic surveying affecting scallop conditions during warmer months, and warrants additional study to determine the cause of the reduction in relative mass of the adductor during warm water experiments.

Conclusion

The present study represents a substantial advancement of the current knowledge regarding the effects of seismic exposure on lobsters and scallops and, more broadly, of marine invertebrates in general. It is one of the first studies to report significant impacts on marine invertebrates resulting from exposure to seismic air gun signals and is perhaps the most comprehensive approach to date. The breakthroughs reported in this study can be attributed to several aspects of the experimental approach and design. First, the exposure was conducted in the field using an air gun, rather than in a tank or with recordings of signals played over an acoustic projector. While this has been discussed in depth previously, it cannot be overstated how important this aspect is to delivering results that apply to real world scenarios. Second, this study approached the experiment with an aim to emulate seismic surveys in terms of exposure levels, exposing experimental animals to levels equivalent to those of a full scale array passing within a few hundred meters. In doing so, the results are scientifically robust and can be extrapolated to understand the effects of exposure in a real survey, without the confounding effects of unrealistic exposure regimes, such as exposing organisms at a range of < 1 m. Third, the design utilised physiological measurements from animals held in controlled conditions, a level of scientific rigour that is crucial for documenting chronic issues.

In lobsters, the key findings of the present study were:

- Exposure to air gun signals did not result in any mortality in any of the experiments comprising this study.
- Two reflexes, tail extension and righting, showed a response following air gun exposure. Tail extension, a simple reflex, was reduced in lobsters from one of the four experiments conducted in this study (summer 2015, 150 in^3 standard pressure experiment) to the day 14 sample time, with warm summer conditions potentially playing a role. The ability to right, a complex reflex, was compromised in three of the four experiments (winter 2013, 45 in^3 standard pressure experiment, winter 2014, 150 in^3 low pressure experiment, summer 2015 150 in^3 standard pressure experiment), with the effect persisting to 120 days post exposure in all experiments and to 365 days post-exposure in the one experiment conducted that long.
- Damage to the sensory hairs of the statocyst, the primary mechanosensory and balance organ of the lobster, was observed following exposure in three of four experiments. This damage was statistically correlated to the delays in righting time.
- Lobsters collected from a site subject to high levels of anthropogenic aquatic noise, relative to the remote site most lobsters were collected from, showed substantial damage to the statocyst prior to the experiment. Exposure did not result in additional damage.
- Haemolymph biochemistry showed little effect from exposure. Assays of 23 electrolytes, minerals, metabolites, organic molecules and enzymes showed no effect resulting from exposure. Haemolymph pH levels were similarly unaffected. Refractive index of the haemolymph indicated a decrease in nutritional condition in several experiments, with the effect lasting until the day 120 sampling point.

- Counts of the number of circulating haemocytes showed a significant reduction in all experiments, with a chronic reduction at day 120 post-exposure in the winter 2014 150 in^3 low pressure experiment. In the same experiment, exposed lobsters sampled 365 days post-exposure showed nearly 2 times the number of haemocytes of control lobsters.
- Embryos exposed to air gun signals and subsequently hatched showed no effect in terms of quantity or quality.

In scallops, the key findings of the present study were:

- Exposure did not cause any incidence of immediate mass mortality, however, repeated exposure significantly increased mortality, and the risk of mortality significantly increased with time as the majority of mortality was recorded at the day 120 sample points.
- Substantial disruptions in the biochemistry of the haemolymph, with a range of electrolytes, minerals and metabolites showing disrupted levels through day 120 post-exposure.
- Haemolymph pH was also significantly affected in two of the three experiments, showing a slight but persistent alkalosis corresponding to exposure level to day 14 post-exposure in those two experiments.
- Scallops demonstrated a reduction of classic behaviours during exposure. Furthermore, air gun signals elicited a novel velar flinch behaviour.
- Scallop reflexes were affected, with exposure resulting in faster recessing times and some indication that righting time may be slowed from the results of one experiment.

Implications

The lobster fishery does not appear to be at risk of mass mortality in direct response to seismic air gun exposure, as evidenced by the lack of mortality or large scale physiological changes. However, a number of concerns over lobster health and ecology over the long term remain. First, disruption of the number of circulating haemocytes can compromise immune function, potentially increasing the risk of disease. As the lobsters in this study were held in optimal conditions – low density, controlled water conditions, easy access to high quality food – their exposure and susceptibility to pathogens was unlikely to reflect that of lobsters in the wild. Along the same lines, the damage incurred to the statocyst and the compromised reflexes following exposure may have an ecological impact that was not investigated in the present study. If statocyst damage results in impairment of the escape response of exposed lobsters, either through a reduction in the capacity to control their escape trajectory or through a reduced ability to position themselves for a subsequent attempt, they may be subject to increased predation. Given the gregarious nature of lobsters and their reliance on aggregative group defence, impaired anti-predator behaviours could have an impact beyond the individual level. Finally, the effects of prolonged or repeated exposure were not assessed in this study. As commercial seismic surveys are conducted over a much longer time period than in this study, lobsters in the wild will likely be subjected to signals over a longer time

period and survey sites may be revisited periodically in the case of 4-D surveys. If the impacts to the statocyst or reflexes are additive, potential impacts would be exacerbated.

Seismic exposure of lobster embryos does not appear to affect development or the hatching of larvae, which reduces some of the concerns over recruitment in the lobster fishery. However, the present study examined exposure at only one life history stage, and further investigation of other stages (e.g. late in embryonic development, phyllosoma, puerulus) are required to develop a comprehensive understanding of the impacts at the population level.

In scallops, the scope, scale and persistence of physiological disturbances suggest that seismic surveys have the potential to severely impact the fishery. Although mass mortality was not observed in the present study, the loss of the capacity for homeostasis raises concerns that the introduction of any further insult (i.e. dredging, warm water conditions, predation stress, etc.) may serve as a tipping point that results in a large scale die-off. Without a mechanistic understanding of the osmoregulatory issues observed in this study, it is difficult to predict how scallops will fare outside of the controlled conditions of an aquaculture facility.

Similar to lobsters, the observed alterations in scallop behaviour and reflexes may have ecological implications that require further investigation to quantify. The inability to right may have implications following disturbance, either through fishery activities or predator-prey interactions. It is unclear how the changes in recessing time might affect scallops, as, initially, faster recessing would appear beneficial. However, this may be energetically demanding in the case of repeated disturbances. In addition, if this change to the recessing reflex is symptomatic of some other issue, such as damage to the statocyst or abdominal sense organ, there may be further ecological ramifications. As in lobsters, the changes observed in haemocyte counts indicate chronic compromised immunity, as the number of circulating haemocytes collapsed in the 120 day samples in both the 2013 and 2014 experiments.

The present study only examined adult scallops, so additional study into other life history stages is required to develop a comprehensive understanding of the impacts of seismic exposure. Larvae may be particularly susceptible, but the behavioural modifications observed in adults have the potential to be more severe in spat, as younger scallops tend to be more active.

Recommendations

At this stage, the gaps in knowledge regarding the effects of exposure to air gun signals are substantial enough that definitive recommendations remain elusive. It is necessary to gain a better understanding of the physiological impacts observed in lobsters and scallops in the present study. Although haemocytic and biochemical disruptions were observed, it was not within the scope of this study to investigate the mechanistic underpinnings of these impacts. An understanding of these mechanisms is critical to fully understand the scope of the impact and the subsequent risk to the fisheries. Specifically, investigating the mechanisms behind the damage incurred by the lobster statocyst, both in terms of the damage resulting from air gun

exposure in naïve lobsters and the pre-existing damage observed in the Taroona reserve lobsters, will help characterise how different types of aquatic noise (e.g. intense, episodic sources like air guns and pile driving *versus* constant sources like ship noise) affect marine organisms. In scallops, the results indicate a severe disruption to their ability to osmoregulate. Given that scallops do not have a well-developed capacity for osmoregulation, this result is surprising and requires a focused study to characterise.

The ecological impacts of the observed results were not investigated as a part of this study, but are necessary to put the results into context. In lobsters, it is not clear whether a damaged statocyst and impaired reflexes might disadvantage lobsters in the wild. Experiments investigating predator avoidance, foraging success and interspecific competition are necessary to evaluate the ultimate impacts of exposure.

In the case of the scallop results, given the scale and duration of physiological and behavioural disturbances, it is critical to develop a better understanding of the long term effects and the impact of additional stressors not present in the controlled conditions of this study (e.g. predation, fishing, abiotic influences). It is conceivable that the severely compromised physiological condition of the scallops exposed to air gun signals in this study were at considerable risk to other stressors, such as dredging; warm water conditions; pathogen outbreak; and over the longer term, factors including rising sea temperatures and ocean acidification, and that a further insult could serve as a tipping point resulting in mass mortality. However, further investigation is needed to either confirm or refute this hypothesis.

The results from the lobster experiments indicate that different life history stages have different levels of sensitivity to air gun signals. As such, it is necessary to evaluate across the life history of an organism to draw broad conclusions over the effect of exposure. Our results indicate that embryos were wholly resistant to exposure and adults showed moderate responses. How early developmental stages such as phyllosoma or puerulus might be affected is not clear and cannot be inferred as they differ morphologically and physiologically from the embryo stages tested here. Similarly, only adult scallops were tested in this study, leaving the impact on scallop larvae unknown. Additional research in this area is critical for forming an ecosystem level understanding of the effects of air gun signals in the marine environment.

As the present study focused on emulating the exposure level of real world, full-scale seismic surveys to determine whether lobsters and scallops demonstrated a physiological response, distance and intensity thresholds were not directly investigated. The use of several different air gun configurations resulted in a limited range of sound exposure level intensities, however, it is apparent from the results that if a threshold exists, the exposure levels used here exceeded it. Given that the results presented here demonstrate that both lobsters and scallops were moderately to severely affected, a determination of threshold levels is now appropriate and would be valuable for estimating the scope of potential effects resulting from exposure as a part of the environmental impact assessment process for seismic surveys. Finally, it is important that the present study is considered a first step into understanding the impacts of seismic surveys on marine invertebrates, not as an endpoint, and that the work carried out here had almost no preceding reference point. Clearly, a number of important questions

remain, and we recommend that they be tackled with the same robust experimental approach as that adopted here, as without this any studies are unlikely to detect the sub-lethal effects of seismic exposure highlighted in this study.

Extension and Adoption

At the request of FRDC the results of the project have been held in confidence until submission of the final report, limiting the ability to disseminate information. However, over the course of the project, the following details have been communicated to stakeholders:

30 October 2012 – UTAS and Origin released media statements relating to the project, which were picked up by radio and print media across the country (see below).

2013 – Jayson Semmens had significant contact with Origin staff, fishing industry representatives and Federal and State Government representatives (AFMA, Tasmanian DPIPWE and Victorian DPI) throughout the year.

7 August 2013 – Rob McCauley presented a 15 minute talk on seismic impacts on marine fauna, with a focus on rock lobster, at a Western Rock Lobster Association meeting at Dongara, WA.

15 August 2013 – Rob McCauley presented a 5 minute overview of the experiment at the 3rd international conference "Effects of Noise on Aquatic Life" in Budapest, Hungary.

02 September 2013 - Rob McCauley and Chandra Salgado Kent presented at the APPEA 2013 Annual Conference, Crown Casino Perth, summarising the FRDC project aims

24 October 2013 – Rob McCauley gave a 15 minute presentation on the FRDC project at the NOPSEMA workshop in Perth, WA.

2014 – Jayson Semmens had significant contact with Origin staff, fishing industry representatives and Federal and State Government representatives (AFMA, Tasmanian DPIPWE, Victorian DPI, DAFF and Tasmanian House of Assembly parliamentarians) throughout the year.

28 August 2014 – Rob McCauley represented the program at a meeting regarding impacts of seismic surveys with the Pearl Producers Association and WAFIC in Fremantle, WA.

27 December 2014 – Rob McCauley presented a summary of seismic impacts, including an overview of the aims of this project, at an ASBTA meeting in Port Lincoln.

2015 – Jayson Semmens had significant contact with Origin staff, fishing industry representatives and Federal and State Government representatives (AFMA, Tasmanian DPIPWE, Victorian DPI, DAFF) throughout the year.

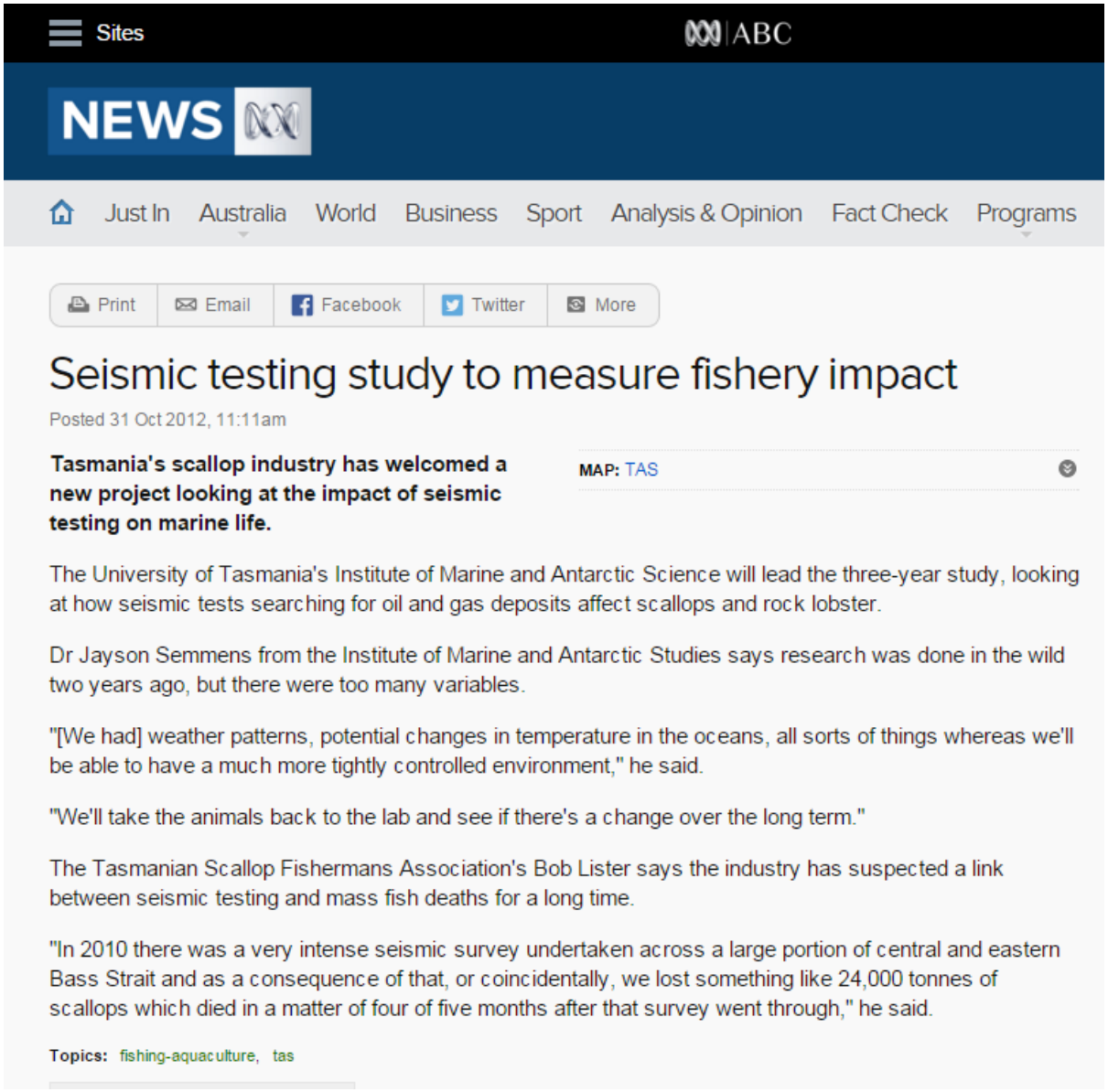
2015 - Rob McCauley had regular informal contact with fishery groups: (Pearl Producers Association, Western Australia and Australian Southern Bluefin Tuna Association (ABSTA); APPEA; and regulators (WA State Government and NOPSEMA) throughout the year.

09 March 2015 - Rob McCauley presents project summary at an Australian Hydrographic Society meeting, Perth, WA

18 March 2015 - Rob McCauley presents a summary of experiments at an underwater noise workshop held for Western Australian Government regulators, WA Fisheries and NOPSEMA, at DEC offices, Bentley, WA.

7 March 2016 – A journal article entitled "Seismic air gun exposure during early-stage embryonic development does not negatively affect spiny lobster *Jasus edwardsii* larvae (Decapoda:Palinuridae)" in the Nature Publishing Group journal Scientific Reports. The paper was accompanied by a media release published by ABC and radio interviews with Jayson Semmens.

Project coverage



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Seismic testing study to measure fishery impact

Posted 31 Oct 2012, 11:11am

Tasmania's scallop industry has welcomed a new project looking at the impact of seismic testing on marine life.

MAP: TAS

The University of Tasmania's Institute of Marine and Antarctic Science will lead the three-year study, looking at how seismic tests searching for oil and gas deposits affect scallops and rock lobster.

Dr Jayson Semmens from the Institute of Marine and Antarctic Studies says research was done in the wild two years ago, but there were too many variables.

"[We had] weather patterns, potential changes in temperature in the oceans, all sorts of things whereas we'll be able to have a much more tightly controlled environment," he said.

"We'll take the animals back to the lab and see if there's a change over the long term."

The Tasmanian Scallop Fishermans Association's Bob Lister says the industry has suspected a link between seismic testing and mass fish deaths for a long time.

"In 2010 there was a very intense seismic survey undertaken across a large portion of central and eastern Bass Strait and as a consequence of that, or coincidentally, we lost something like 24,000 tonnes of scallops which died in a matter of four of five months after that survey went through," he said.

Topics: fishing-aquaculture, tas

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Spiny lobster eggs not impacted by underwater oil and gas exploration air guns, study finds

By Pablo Vinales

Updated Wed at 10:33am

PHOTO: The study was carried out on in Tasmania's Storm Bay. (Supplied: IMAS)

There is no impact of the embryonic development of spiny lobsters from seismic air guns used in underwater oil and gas exploration, a new study has found.

RELATED STORY: Climate change driving species to the poles faster than predicted: scientists

RELATED STORY: Commercial rock lobster quota to remain

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MAP: TAS

The study, carried out by Institute for Marine and Antarctic Studies (IMAS) and Curtin University, researched whether compressed air guns used to explore sub-seafloor deposits adversely affected the development of spiny lobster embryos.

IMAS principal investigator Jayson Semmens said whilst the results were from early stage embryos, the findings were reassuring.

"That's good news for the lobster industry, but also for the oil and gas industry," he said.

"They don't want to impact fisheries or environments but the important caveat is that this is just one stage."

Key points:

- Research examined effect of air guns on young lobsters
- The air guns are used by miners searching for oil and gas deposits
- Scientists found there was no effect on the crustaceans

The researchers tested three different air gun configurations at various distances on egg-carrying female spiny lobsters on a shallow reef in Storm Bay, in southern Tasmania.

"The way we tested it is we exposed the females carrying eggs to these airguns in the field and brought them back in tanks and we kept them in captivity until their eggs hatched into larvae," Mr Semmens said.

"And then we tested those larvae to see what their abilities were and see if there was any change in their behaviour and it turned out that there was no difference."

The research is one of the first in the world to look at the impacts of noise seismic air guns cause to marine invertebrates like lobsters.

PHOTO: The study measured the effects of seismic air guns on embryonic lobster larva. (Supplied: IMAS)

It is part of a four-year research program looking at seismic testing to establish best practice procedures between government, research organisations and industry.

Topics: science-and-technology, animal-science, marine-biology, tas

First posted Wed at 6:36am

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Appendix I: Sample sizes

Table 20. Sample sizes from lobster experiments. The larval development experiment lobsters were sampled at day 365 of the winter 2104 150 in^3 low pressure experiment (indicated with an asterisk). The larval development experiment lobsters were sampled at day 120 of the winter 2014, 150 in^3 standard pressure experiment (indicated with a dagger). Lobsters sampled at days 2 and 14 in the winter 2014, 150 in^3 standard pressure were serially sampled.

	Exposure	Winter 2013, 45 in^3 standard pressure				Winter 2014, 150 in^3 low pressure					Winter 2014, 150 in^3 standard pressure				Summer 2015, 150 in^3 standard pressure			
		Day 0	Day 2	Day 14	Day 120	Day 0	Day 2	Day 14	Day 120	Day* 365	Day 0	Day 2	Day 14	Day† 120	Day 0	Day 2	Day 14	Day 120
Mortality, reflexes, Statocysts, HPI, THC, pH, Refractive index,	C	11	10	10	11	9	9	8	8	7	–	11♂ 11♀		11♀	–	12	14	–
	E	10	10	11	10	8	8	8	7	10		10♂ 10♀		10♀		15	13	
Biochemistry assays	C	–	–	–	–	9	9	8	8	–		11♂	11♂		–	–	–	–
	E					8	8	8	7			11♀	11♀					
Larval development, quantity, quality	C	10				7					11				–			
	E	10				10					10							

Table 21. Sample sizes from scallop experiments.

	Exposure	2013			2014			2015	
		Day 0	Day 14	Day 120	Day 0	Day 14	Day 120	Day 14	Day 120
Mortality, THC, pH, Refractive index, Indices	0-pass	16	16	17	20	17	16	19	All scallops dead upon recovery
	1-pass	19	17	15	19	16	12	–	
	2-pass	17	20	16	17	15	15	–	
	4-pass	20	19	14	19	16	12	17	
Behaviour	0-pass	–			19			12	
	1-pass				16			–	
	2-pass				8			–	
	4-pass				8			7	
Recessing	0-pass	–	32	–	–	38	16	32	–
	1-pass		32			33	12	–	
	2-pass		34			37	15	–	
	4-pass		33			38	12	33	
Righting	0-pass	–	–	–	–	–	–	37	–
	1-pass							–	
	2-pass							–	
	4-pass							32	
Biochemistry assays	0-pass				20	17	15		
	1-pass				19	15	15		
	2-pass				18	16	15		
	4-pass				18	15	13		

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