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Anthropogenic noise causes body malformations and delays development in marine larvae

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Understanding the impact of noise on marine fauna at the population level requires knowledge about the vulnerability of different life-stages. Here we provide the first evidence that noise exposure during larval development produces body malformations in marine invertebrates. Scallop larvae exposed to playbacks of seismic pulses showed significant developmental delays and 46% developed body abnormalities. Similar effects were observed in all independent samples exposed to noise while no malformations were found in the control groups (4881 larvae examined). Malformations appeared in the D-veliger larval phase, perhaps due to the cumulative exposure attained by this stage or to a greater vulnerability of D-veliger to sound-mediated physiological or mechanical stress. Such strong impacts suggest that abnormalities and growth delays may also result from lower sound levels or discrete exposures during the D-stage, increasing the potential for routinely-occurring anthropogenic noise sources to affect recruitment of wild scallop larvae in natural stocks.

There is growing concern about the impact that noise from human activities may be having on marine fauna[1]. However, we are still far from understanding how noise affects marine animals either at the individual or population levels. Much of what is known about the effects of noise comes from experiments with terrestrial animals. These studies show that, while some physiological and behavioural responses are recoverable[2–4], other effects such as alteration of DNA or gene expression and tissue damage to vital organs are irreversible[5,6]. In marine fauna, moderate noise levels are known to provoke startle or avoidance responses in many taxa[1,3], and to increase metabolism and reduce growth and reproductive rates in brown shrimp (*Crangon crangon*)[7]. High noise levels have been reported to damage the auditory system of fish and cephalopods[3,8] and cause hearing loss in dolphins[9]. In the wild, geophysical seismic surveys have been singled out as the cause for atypical mass strandings of giant squid (*Architeuthis dux*) with extensive tissue damages[10] while navy sonar have been implicated in the mass strandings of some whale species[11,12]. But much less is known about the effect of noise on early developmental stages of marine life and results vary widely. For example, exposure to a single discharge of an array of seismic airguns did not seem to affect crab larvae survival[13] while exposure to a single discharge of a close airgun can increase mortality in some fish larvae[14]. It is currently unclear whether there are ontogenetic variations in the vulnerability of animals to noise, but an enhanced sensitivity during juvenile stages could have important consequences on populations by decreasing recruitment.

Even though the mechanisms remain cloudy, incidental noise exposure may be already impacting important food resources. Fishermen worldwide complain that seismic surveys produce economic loses by reducing captures of a wide range of commercial species[15]. Their concerns include potential delayed effects in species abundance if noise affects reproductive or developmental stages (e.g. http://www.abc.net.au/rural/tas/content/2012/08/s3576796.htm). But studies on the impact of seismic surveys on fishing captures have reported variable results, from no catch changes in non-mobile species such as shrimp[16] to reductions of 70% in the catch rates of mobile and valuable fin-fish species such as cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*)[15,17]. Despite uncertainties about how noise may affect marine fauna and fisheries, several countries have already implemented regulations that reduce overlap between seismic surveys and fishing activities. However, these regulations do not address concerns that noise might affect stock recruitment and thereby cause delayed reductions in catch rates.

Here we demonstrate that exposure in a tank to low frequency sounds like those produced by seismic surveys disrupts the larval development of a marine invertebrate, the New Zealand scallop (*Pecten novaezelandiae*), with potential consequences on recruitment. We discuss possible mechanisms for these effects in marine invertebrate larvae and show that comparable exposure levels could occur incidentally in the wild from widely-used high power anthropogenic noise sources.

Results

We analysed 4881 scallop larvae from a larger sample of fertilized eggs divided into four noise and four control sub-samples. The noise samples were exposed to a playback of pre-recorded seismic airgun sounds at 3 s intervals. Larvae were sampled from both groups at seven fixed intervals between 24 and 90 hours after fertilization. During this period we observed their development through gastrula, trocophore, early veliger and advanced D-veliger larval phases (Supplementary Table). In the first sample, 24 hours after the start of noise exposure, all larvae were in gastrula and trocophore stages. The average proportion of larvae in the most advanced stage (trocophore) was significantly higher (42%) in the control group than in the noise group (17%) (Fisher exact test p = 0.0002, n = 800 larvae, 100 larvae examined from each sub-sample). This delay in development continued to be evident in the following sampling events in spite of the variability introduced by when the sampling moments fell with respect to natural development transitions (Figure 1 and Supplementary Table).

Malformations were observed in all flasks of the noise-exposed group starting in the sample corresponding to 66 hours post-fertilization. By this time, 100% of the larvae in the control group had transitioned to D-veliger stage (Figure 1 and Supplementary Table). At experiment completion, after 90 hours, all 329 larvae sampled in the control group were normal D-veliger, while an average of 46% of the noise-exposed larvae (27 to 91% in the four flasks of the noise group) showed malformations (63 out of 141 sampled larvae, see Supplementary Table). These malformations were evident as abnormal growth, with localised bulges in the soft body of the larvae, but not in the shell.

Discussion

The objective of this study was to determine if noise exposure during larval development can cause body malformations in marine fauna. The results were not subtle: high proportions of malformed larvae were found in all four containers exposed to noise while no malformations were found in the four control flasks. Also, significant developmental delays were evident beginning at the first sampling event after 24 hours of exposure. All eight containers were isolated throughout the experiment and, apart from the noise exposure, were treated identically. Thus the experiment provides a robust indication of the potential consequences of high level sound exposure during larval development. This is, to our knowledge, the first evidence that sound can cause growth abnormalities in larvae. The abnormalities observed here are comparable to those caused by chemical pollutants or water acidification, which have a clear impact on larval survival[18]. We therefore conclude that if larvae in the wild are subject to intense noise exposure during development, this could reduce recruitment and so have a delayed impact on stocks of mature animals.

Although noise has been shown to affect the behaviour and physiology of animals in a variety of ways, including disruptions in the neuroendocrine, cardiovascular and immune systems[5], very few studies
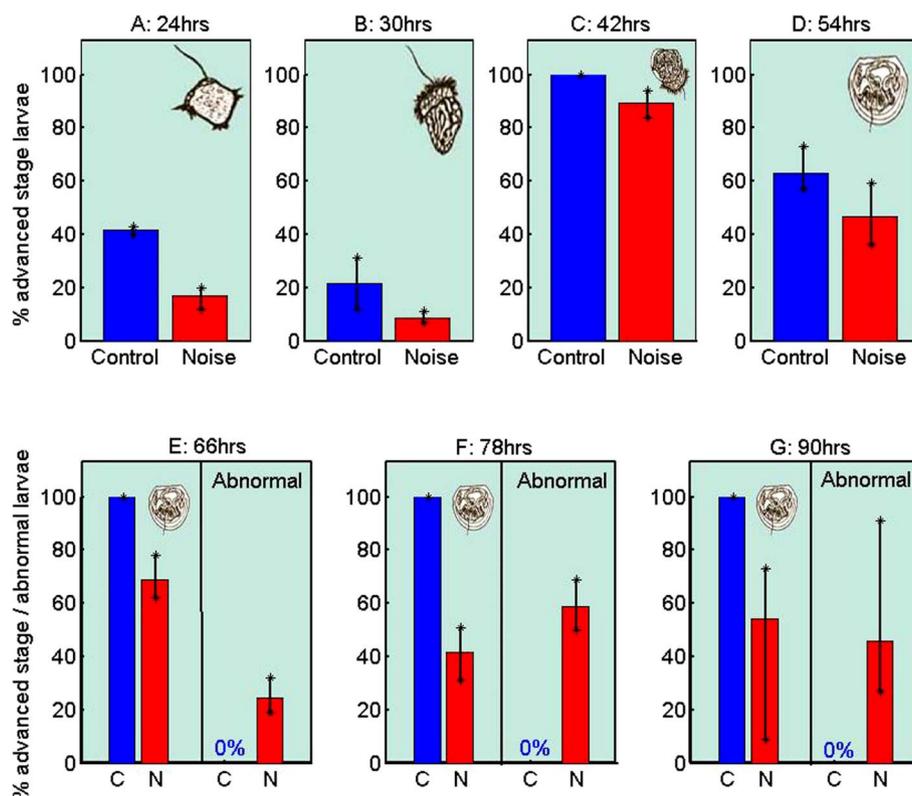

Figure 1 | Comparative results for the control (C) and noise (N) groups. The height of the bars indicates the mean proportion of abnormal larvae (with body malformations) and of larvae in the most advanced developmental stage observed for each sampling interval: (A) trocophore; (B) flagelated early veliger; (C) newly secreted straight-hinged D-veliger; (D), (E), (F), (G) straight-hinged D-veliger mixed with some pediveliger in (G). The error bars mark the minimum and maximum values observed for each condition. Sample size was 800 larvae per sampling in (A) to (D) and 738, 473 and 470 larvae in (E), (F) and (G), respectively. Larval schematics reproduced with permission from FAO (2004) Helm, Bourne and Lovatelli. The hatchery culture of bivalves, a practical manual. http://www.fao.org/docrep/007/y5720e/y5720e0a.htm.

have linked growth malformations to noise exposure for any animal. A possible mechanism for the abnormal bulges observed here in scallop larvae involves morphogenetic changes mediated by homeobox genes. There are no studies of noise-induced stress influencing homeobox genes, but these genes are implicated when cells respond to a miscellanea of other stressors (e.g. oxidative, thermal, hydric), and these responses include mediating tumour control or progression[19]. An alternative mechanism might relate to system-wide calcium deregulation, which has been linked to noise-induced physiological stress in pregnant rats exposed to noise, giving birth to offspring with greater fluctuating asymmetry or lower dental calcification[20]. Although the exact mechanism is far from evident, physiological stress is likely the mediator for the developmental delays and growth abnormalities reported here. Particular vulnerability in the D-veliger stage might relate to the progressive calcification of the shell creating a sound impedance gradient in the body which increases mechanical stress concentration in the adjacent tissues. While behavioural responses to noise can vary widely among and within species, stress response mechanisms that operate at the genetic, cellular or physiological level are more likely to be conserved across taxa[4], suggesting that other invertebrates with similar growth patterns may be similarly affected. Thus, identifying the mechanisms by which noise induces the developmental defects observed here will be critical to predict the impact of different exposures, to assess whether other species may also be affected, and to mitigate these effects.

The small size of scallop larvae and the absence of strong tissue density gradients in early development phases lead us to propose that the observed damage is related to particle motion rather than to the pressure component of the noise exposure. In the far-field of an acoustic source the pressure and velocity components are related as $p = v*z$ (z = characteristic impedance of the medium)[21]. In this experiment, larvae were in a tank in the near-field of the acoustic transducer and so would likely have experienced higher particle motions than would be expected at the same pressure level in the far-field. Intense underwater sound sources such as airguns, pile driving, sonar and blasting have back-calculated peak source levels ranging from 230 to, in the case of blasting, >300 dB re 1 μPa at 1 m[22]. These activities routinely ensonify large areas with sound pressure levels higher than the 160 dB$_{rms}$ re 1 μPa received by the scallop larvae in this experiment. For example, a seismic array with an equivalent source level of 260 dB pk-p re 1 μPa at 1 m[22] will produce levels in excess of 160 dB$_{rms}$ (e.g., using a 97% energy window around each pulse to measure RMS level) over hundreds of km-squared assuming spherical spreading of sound[21].

But the particle velocities experienced by the larvae here (about 4–6 mm s^{-1} RMS) imply higher far-field pressure levels of some 195–200 dB$_{rms}$ re 1 μPa, reducing the potential impact zone. However, there are several reasons why larvae in the wild may be impacted over larger distances than these approximate levels suggest. Given the strong disruption of larval development reported here, weaker but still significant effects can be expected at lower exposure levels and shorter exposure durations, especially if some ontogenetic stages such as the D-veliger prove to be particularly sensitive. Moreover, the low frequency sounds tested here propagate in complex sound fields in which convergence zones and re-radiation of sound transmitted through the sea-floor[19] can create regions with high sound levels far from the source[23]. The sound field experienced by an organism is a complex function of its location with respect to the sound source and acoustic boundaries in the ocean necessitating *in situ* measurements to establish the precise exposure level. This, and the inaccuracies arising from measuring the sound field in a small tank as in our experiment, makes it difficult to predict if larvae were subjected to higher exposures than might arise from anthropogenic noise sources in the oceans. It will be important in future work to establish the thresholds of exposure level and duration that lead to growth abnormalities.

Short exposures to intense seismic signals are known to increase mortality of fish larvae at short ranges[14]. Our results show unequivocally that longer exposures to lower levels cause delayed development and abnormal growth of scallop larvae. Both effects will reduce the probability of larval survival in the wild by increasing exposure to pelagic predators or by reducing larval competence[18,24]. There are no *a priori* reasons to expect that scallop larvae are more vulnerable to sound than other shellfish with similar larval development and the effects of noise on larvae of other species therefore requires examination. Shellfish and other invertebrates form the base of the trophic-web in the oceans, providing an important food source for fish, marine mammals and humans. Invertebrate fisheries yield annually 12 million tons of catches and have grown by 6 fold since 1950[25], a period during which other human activities have also increased dramatically in many areas of the oceans[1]. Overfishing and coastal development may act synergistically to threaten shellfish populations. Here we propose that noise exposure during critical growth intervals may also contribute to stock vulnerability, underlining the urgency to investigate potential long-term effects of acoustic pollution on marine fauna. Moreover, these results call for applying the precautionary principle when planning activities involving high-intensity sound sources, such as explosions, construction or seismic exploration, in spawning areas of marine invertebrates with high natural and economic value.

Methods

New Zealand scallops were collected by divers from a scallop bed located 1 km offshore and at 15 m depth on a sandy bottom. Scallops were transported in a gently aerated tank to the Leigh Marine Laboratory (University of Auckland) some five nautical miles from the scallop bed. Once in the laboratory, the scallops were maintained in a tank with running seawater at 21°C and fed with a mix of microalgae (T-*Isochrysis galbana*, *Pavlova lutherii* and *Dunaliella tertiolecta*) for two days before the experiment. Individuals with mature gonads were selected by visual inspection and induced to spawn by placing the scallops in a warm (~23°C) suspension of concentrated microalgae in seawater. New Zealand scallops are simultaneous hermaphrodites and white sperm release is eventually followed by release of bright orange eggs. Once a scallop started producing sperm it was transferred sequentially to separate 1 litre containers with UV-1 μm filtered seawater to obtain gametes clean of contamination from sperm of other individuals or self-fertilized eggs. Sperm from three scallops was mixed and used to fertilize eggs from a fourth individual.

Samples of the fertilized eggs with a concentration of ~350 eggs ml^{-1} were collected and stored in eight 60 ml water-tight polyethylene flasks filled with UV-1 μm filtered seawater. Four flasks were randomly selected for noise exposure and the remaining four flasks formed the control group. Noise flasks were placed in a thin plastic mesh and suspended at 1 m depth in a 2 m diameter by 1.3 m water depth tank filled with seawater at 21°C and with the bottom covered by sand. Control flasks were located in the same way and at the same time in an adjacent second tank with the same characteristics and seawater. The four noise flasks were suspended 5 to 10 cm in front of a J-9 sound transducer emitting a pulse once every 3 s (Figure 2). Noise exposure started immediately after the jars were placed in the tank, within one hour after fertilization. The control flasks received no sound playback. The noise and control flasks were sampled at fixed times post fertilization (24, 30, 42, 54, 66, 78 and 90 h). Samples were gathered by pipetting 800 μL from each flask after gently mixing the flask to suspend all larvae in the water column. After taking the samples at 66 h, 25 ml of each flask were interchanged for oxygenated UV-1 μm filtered seawater to improve the rearing conditions for larvae. This reduced the larval concentration in all flasks. All samples were fixed with formaldehyde (5%) and analysed using a Sedgewick Rafter counting microscope slide. The developmental stage of the first 100 larvae encountered in random transects of the counting reticule was determined. Fertilized scallop eggs undergo complete and heteroquadrantal spiral cell division to form a blastula, which through epiboly and invagination progresses to a spherical gastrula[26]. The non-motile gastrula develops into a motile trochophore larva, which then changes into the early veliger stage soon after developing a velum and shell. Further development leads to the D-veliger, the straight-hinged shell larval stage, which later becomes a pediveliger after developing a foot with which to crawl[26].

Our sampling intervals were fixed and so did not precisely track the changes in developmental stage of the larvae. The developmental stages observed in samples were gastrula, trochophore, early veliger and D-veliger. Because only two developmental stages were observed in each sampling event, we used 2 × 2 contingency tables and applied a Fisher exact test to investigate if the proportion of larvae in the most advanced state, or showing abnormalities, was different for the control and noise groups. Statistical tests were performed on results from the first and last sampling intervals only to avoid serial correlation. The first sampling interval was tested
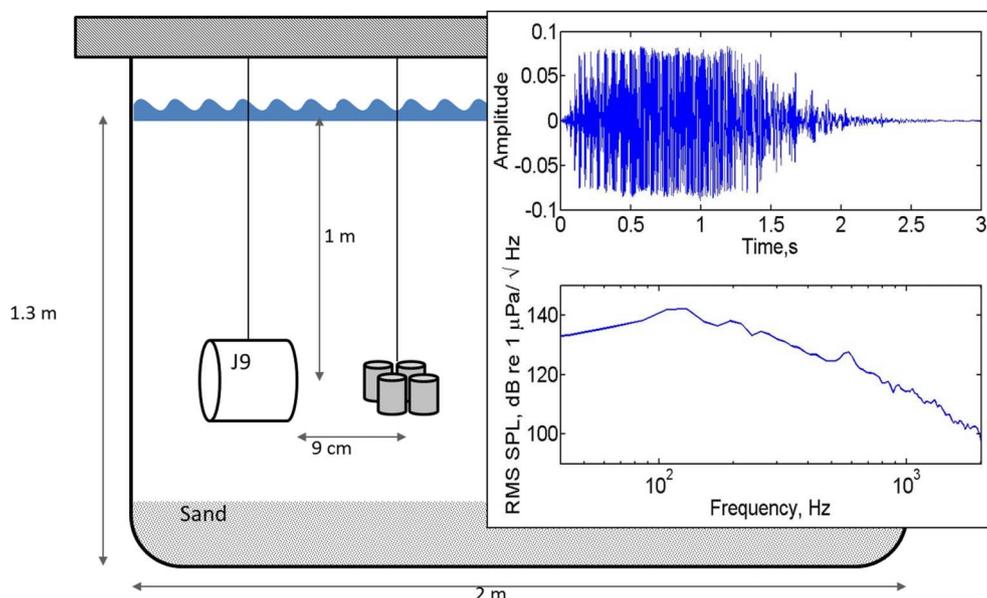
Figure 2 | Diagram of the experimental set-up in the tank and characteristics of the noise exposure received by the scallop larvae, showing the waveform and the power spectrum level of an individual pulse (1024 point FFT, 50% overlap, 2048 Hanning window).

for developmental delays while the last interval was tested for the presence of abnormalities. Because the results at each sampling interval are influenced by when the sampling moments fell with respect to the development transitions, no test was made as to whether delays accumulated over the exposure. A bootstrap analysis (1000 iterations) was performed to test the significance of the percentage of larvae in the most advanced stage for the control and noise treatments at the first sampling point. R-Studio Inc (c) was used for the statistical analysis.

Sound exposure. Seismic pulses were recorded in June 2001 in deep waters off W Ireland, at tens of kilometres from a seismic survey vessel with a 6920 cubic inch array. The recording system was a 300 m long towed hydrophone array (EcologicUK) equipped with two Benthos AQ-4 hydrophones and a high-pass filter at 200 Hz, resulting in an approximately flat response between 200 Hz and 22 kHz. A seismic pulse from this recording was extracted and repeated to construct a sound file with an inter-pulse interval of 3 s. The pulse was filtered (single pole at 40 Hz and a zero at 200 Hz) to partially correct for the frequency response of the recording system. This file was played with a J9 transducer with an approximately flat frequency response from 40 Hz to 20 kHz suspended at 1 m depth in the experimental tank.

The sound pressure level (SPL) received by the larvae was measured at the position of the closest and furthest flasks from the J9 transducer with a HTI-96 hydrophone (sensitivity of -165 dB re: 1 V/µPa) recording to a calibrated Edirol 09HR digital recorder, with an overall frequency response of 20 Hz to 22 kHz. The recorder was calibrated in the laboratory using a signal generator and oscilloscope. The same recording system was used to measure the experimental signal received by the larvae and the background noise in the tanks in absence of exposure. The broadband (20 Hz–22 kHz) background noise was 132 dB re 1 µPa RMS in the control tank and 131 dB re 1 µPa RMS in the noise tank between consecutive pulses.

Because larvae were within the near-field of the J9 transducer and in a relatively small tank, both the SPL and the particle velocity are required to define the noise exposure. We made a rough approximation of the particle acceleration at the location of the samples in the tank by measuring the pressure gradient between two HTI-96 hydrophones[27]. The two hydrophones were separated by 3 cm (between acoustic centres) and the pressure gradient was measured in three axes, with each measurement repeated in each axis with the hydrophones reversed to assess the effect of phase differences between the hydrophones. The RMS (square root of mean squared) acceleration was calculated for each axis and then combined as an RMS over the axes to estimate the magnitude of the triaxial acceleration vector. Particle velocity was computed for each axis as the integral of the acceleration vector, and the RMS velocities in the three axis were combined to estimate the magnitude of the triaxial particle velocity. The far-field pressure that would be required to produce this velocity was estimated using $p = v*z$ ($z =$ characteristic impedance of the medium, i.e., 1.5 MRayls for seawater)[21]. The received pulse at the location of the noise flasks had a -3 dB frequency band from 86 to 129 Hz. The duration of the window containing 97% of the pulse energy was 1.4 s. Over this window the received sound pressure level was 160 to 164 dB RMS re 1 µPa, corresponding to a sound exposure level (SEL) of 161 to 165 dB RMS re 1 µPa²s. The 3-axis RMS acceleration was 3 to 4 m s^{-2} and the 3-axis RMS particle velocity was 4–6 mm s^{-1}. The results for the reversed hydrophones differed by less than 1 dB. Although these values were measured repeatably, the sound field in a small tank can vary widely over short distances. Thus, the SPL and particle motion are difficult to measure accurately but our estimates nonetheless provide an indication of the exposure level received by the larvae. The inhomogenous sound field may also lead to some variability in exposure among

flasks. This effect was mitigated by locating the four flasks close together and by interchanging the positions of flasks within each group at random after each sampling event. Thus, the overall exposure received by each flask was likely fairly similar.

1. Slabbekoorn, H. *et al.* A noisy spring: the impact of globally rising underwater sound levels on fish. *Trends Ecol. Evol.* **25**(7), 419–427 (2010).
2. Beckers, O. M. & Schul, J. Developmental plasticity of mating calls enables acoustic communication in diverse environments. *Proc. Roy. Soc. B.* **275**, 1243–1248 (2008).
3. McCauley, R. High intensity anthropogenic sound damages fish ears. *J. Acoust. Soc. Am.* **113**, 638–642 (2003).
4. Popper, A. & Hastings, M. The effects of anthropogenic sources of sound on fishes. *Jour. Fish. Bio.* **75**, 455–489 (2009).
5. Kight, C. & Swaddle, J. How and why environmental noise impacts animals: an integrative, mechanistic review. *Ecol. Lett.* **14**, 1052–1061 (2011).
6. Chang, E. F. & Merzenich, M. Environmental noise retards auditory cortical development. *Science* **300**, 498–502 (2003).
7. Lagardère, J. Effects of noise on growth and reproduction on *Crangon crangon* in rearing tanks. *Mar. Biol.* **71**, 177–185 (1982).
8. André, M. *et al.* Low-frequency sounds induce acoustic trauma in cephalopods. *Front. Ecol. Environ.* **9**(9), 489–493 (2011).
9. Nachtigall, P., Supin, A., Pawloski, J. & Au, W. Temporary threshold shifts after noise exposure in the bottlenose dolphin (*Tursiops truncatus*) measured using evoked auditory potentials. *Mar. Mamm. Sci.* **20**(4), 673–687 (2004).
10. Guerra, A., Gonzalez, A. F., Pascual, S. & Dawe, E. G. The giant squid Architeuthis: an emblematic invertebrate that can represent concern for the conservation of marine biodiversity. *Biol. Conserv.* **144**, 1989–1997 (2011).
11. Frantzis, A. Does acoustic testing strand whales? *Nature* **392**, 29 (1998).
12. Jepson, P. *et al.* Gas-bubble lesions in stranded cetaceans. *Nature* **425**, 575–576 (2003).
13. Pearson, W., Skalski, J., Sulkin, S. & Malme, C. Effects of seismic energy releases on the survival and development of zoeal larvae of dungeness crab (*Cancer magister*). *Mar. Env. Res.* **38**(2), 93–113 (1994).
14. Cox, B., Dux, A., Quist, M. & Guy, C. Use of a seismic airgun to reduce survival of nonnative lake trout embryos: a tool for conservation? *North Am. Jour. Fish. Manag.* **32**(2) (2012).
15. Engás, A. & Lokkeborg, S. Effects of seismic shooting and vessel-generated noise on fish behaviour and catch rates. *Bioacoustics* **1**(2–3), 313–316 (2002).
16. Andriguetto-Filho, J. M. *et al.* Evaluating the impact of seismic prospecting on artisanal shrimp fisheries. *Cont. Shelf Res.* **25**(14), 1720–1727 (2005).
17. Engas, A., Lokkeborg, S., Ona, E. & Soldal, A. Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). *Can. Jour. Fish. Aquat. Sci.* **53**, 2238–2249 (1996).
18. Kadar *et al.* The influence of engineered Fe_2O_3 nanoparticles and soluble iron on the developmental toxicity caused by CO_2-induced seawater acidification. *Env. Pol.* **158**(12), 3490–97 (2010).
19. Tower, J. Stress and stem cells. *Dev. Biol.* **1**(6), 789–802 (2012).
20. Siegel, M. & Mooney, M. Perinatal stress and increased fluctuating asymmetry of dental calcium in the laboratory rat. *Am. J. Phys. Anthropol.* **73**, 267–270 (1987).
21. Urick, R. *Principles of Underwater Sound.* New York: McGraw-Hill, (1983).

22. Hildebrand, J. Anthropogenic and natural sources of ambient noise in the ocean. *Mar. Ecol. Prog. Ser.* **365**, 5–20 (2009).

23. Madsen, P. *et al.* Quantitative measures of air gun pulses recorded on sperm whales (*Physeter macrocephalus*) using acoustic tags during controlled exposure experiments. *J. Acoust. Soc. Am.* **120**(4), 2366–2379 (2006).

24. O'Connor, M. *et al.* Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proc. Nat. Acad. Sci.* **104**(4), 1266–1271 (2007).

25. Anderson, S., Flemming, J., Watson, R. & Lotze, H. Rapid global expansion of invertebrate fisheries: trends, drivers, and ecosystem effects. *PLoS ONE* **6**(3), doi:10.1 371/journal.pone.0014735 (2011).

26. Cragg, S. M. Development, physiology, behaviour and ecology of scallop larvae. In: *Scallops: Biology, Ecology and Aquaculture* (Ed. By Shumway, S. E. & Parsons, G. J.). Pp 45–122 London. UK: Elsevier (2006).

27. Gade, S. Sound Intensity I. *Brüel and Kjær Technical Review* **3**, 3–39 (1982).

Acknowledgements

Author contributions

N.A.S. conceived the experiment, which was designed by her and N.J.D. and carried out by them with the help of S.H. and J.W. N.A.S., J.A. and M.J. set up the acoustic system, calibrated it and characterized the acoustic signals. N.A.S. wrote the paper and all authors contributed to it and to the scientific discussion.

Additional information