

VIA Email

John FitzGerald
Principal Officer
Legislation, Licencing & Property Management
National Parks and Wildlife Service (NPWS)

**Re: Review of Study Addressing Potential Effects of Seismic Surveys on
Zooplankton**

Dear Mr. FitzGerald:

On behalf of the International Association of Geophysical Contractors (IAGC), we provide this letter to address recently published research results suggesting that the compressed air sources used in offshore seismic surveys may adversely affect zooplankton at greater ranges than are documented in existing published studies.¹ McCauley et al. (2017)'s interpretation of limited data samples from the two-day study suggests that zooplankton may experience mortality and/or displacement at ranges exceeding 1000 meters, whereas past studies have typically found effects out to 10 meters or so.² We provide this letter and the attachment to inform the National Parks and Wildlife Service (NPWS) consideration of McCauley et al. (2017) in agency review documents addressing the potential environmental effects of seismic surveys, such as those any being prepared for seismic activities offshore Ireland. We request that NPWS include this letter and the attachment in the relevant administrative records.

Because the McCauley et al. (2017) results were so inconsistent with previously documented effects, we sought and received independent expert reviews of the paper by leading plankton ecologists at well-respected scientific institutions. The written reviews are provided in the attachment to this letter. In short, the reviewers expressed the opinion that although the results of the study should be considered further, the data were not sufficient to support the conclusions offered by McCauley et al. (2017). The following summarizes the observations and criticisms of the independent reviewers.

¹ See McCauley R.D., R.D. Day, K.M. Swadling, Q.P. Fitzgibbon, R.A. Watson, and J.M. Semmens. 2017. Widely used marine seismic survey air gun operations negatively impact zooplankton. *Nature: Ecology & Evolution*, 1(7):195. DOI: 10.1038/s41559-07-0195.

² See references at the end of this letter.

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1. The sample size was inadequate. McCauley et al. (2017) relies upon a control and experimental sample each day on two consecutive days. The fact that the zooplankton density on Day 2 was two orders of magnitude lower (or 1% of) than the densities on Day 1 illustrates the patchy nature of zooplankton distribution. This large disparity of sample sizes on Days 1 and 2 caused the authors to adopt ratio values for the statistical analysis instead of using the raw data. More than half of the samples had zero animals in the net tows, and the samples had to be omitted from the analysis. Additionally, the CTD (conductivity, temperature, depth) profiles on the two days indicated differences in salinity and tide height between the two sets of samples, associated with different species communities and different animal densities on the two days. Moreover, Day 1 and Day 2 results should not have been pooled.

2. Water column movement data were insufficient to support the contention of a “hole” in the plankton field. The reviewers found the single measurement of water column movement to be insufficient. Samples at multiple depths and multiple times should have been taken in order to account for effects of tidal differences and drag from the water-seabed interface in the shallow waters of the test environment. As a result, the reviewers found the laborious attempts to reconstruct the movement of the water mass sampled by the acoustics, and to infer a “hole” in the plankton, to be implausible and insufficient to substantiate claims of effects out to 1 km or more. In other words, the independent reviewers found the presumption of the water moving uniformly at one speed and direction at all depths to be incorrect and, therefore, the “corrected” positions of the acoustic samples were not accurate.

3. Towed net and acoustic survey data disagree regarding zooplankton class size. The independent reviews explain that the acoustic frequency used in the sonar sampling (120 kHz) could not detect the copepods or similar sized zooplankton that were found in the net tows. Conversely, larger adult krill, small fish larvae, and larger classes that were detected by the sonar were not found in the net tows. McCauley et al. (2017) suggested that these large species may not have been present in the water or were able to swim strongly enough to avoid the nets. However, if that were the case, then any larger species that were killed by the sound source (and thus unable to swim away from the nets) would have been found in the nets. But no dead individuals of the large species detected by the sonar were found in the nets after sound exposure.

4. Did the acoustic “hole” indicate dead zooplankton or zooplankton that had swum to the bottom, just 10 meters away? The stronger swimming abilities of larger zooplankton species support the notion that those species were able to swim downward in response to the seismic sound, creating the acoustic appearance of having left but in fact having only exhibited a temporary minor behavioral avoidance response common in zooplankton as predator avoidance behavior. The reviewers noted the presence of a dense acoustic scattering layer in the “after exposure” figures in McCauley et al. (2017) and suggested that this might be the larger krill that were not observed in the upper water column.

5. Bottom sampling should have been conducted. The reviewers suggested that McCauley et al. (2017) should have collected bottom samples, which would have addressed the questions of whether the large zooplankton were present, whether they had been killed and sunk to the bottom, or whether they actively swam to the bottom.

6. The wrong size nets were used and were not towed correctly. The reviewers noted that the small mesh size and small opening of the bongo nets used in this study were inadequate for sampling the larger zooplankton imaged by the acoustics. The reviewers noted that the angled tow at inconsistent speeds may have led to different sample sizes collected by different tows, and mortality could have been induced at the cod end of the net due to “packing” of plankton in the larger sample sizes. One reviewer also noted that fine mesh nets like the ones used in the study can clog with phytoplankton and “reject” zooplankton through part of the tow due to the clog of phytoplankton preventing through-flow. The reviewers suggested that larger nets should have been used, that they should have been towed vertically, and that depth-dependent sampling (*e.g.*, with Nisken, Nansen, Van Dorn, Kimmerer, or similar bottles) should have been used in addition to or instead of nets.

7. There is statistical error in the net tow data. The reviewers questioned the use of each net in a single bongo net tow as a true replicate. The standard procedure would have been to select one of the two nets for quantitative analysis. Had McCauley et al. (2017) followed this standard approach, the statistical sample size would have been lowered from N=12 to N=6, which would have been inadequate to support the statistical analyses offered by the authors. In other words, all “statistically significant” effects noted by McCauley et al. (2017) for the net samples were likely not statistically significant and therefore would likely not have a negative population effect or significant trophic-level impact.³

We have shared these independent reviews with the authors of the McCauley et al. (2017) paper, and those authors have concurred with many of the shortcomings identified by the reviewers. Sponsors are planning follow-up research to further investigate the results reported in McCauley et al. (2017). We expect that those sponsors will collaborate with the McCauley et al. (2017) authors as they undertake that work.

In sum, it is clear that far too much weight has been given by some organizations to the results of McCauley et al. (2017), which purport to show patterns and trends that do not actually exist in the data. For the reasons identified in the independent peer reviews, as summarized above and provided in the attachment, the results presented by McCauley et al. (2017), respectfully, are of questionable scientific merit and, accordingly, must be subjected to more rigorous scientific study before being accepted as the “best available science” regarding the potential effects of seismic sound on zooplankton. Existing published studies demonstrating that any seismic effects on zooplankton occur only to tens of meters remain the best available science

³ Consistent with the observations of the independent reviewers, McCauley et al. (2017) notes that there were plausible alternative explanations for both the net samples and acoustic samples, such as the nets not being towed at an even speed and “back-flushing,” nets not capturing the species that would show up in the acoustic data, animal behavioral response to the acoustic passes that would create the appearance of animals having “disappeared,” and, most importantly, natural fluctuations in the distribution and abundance of the zooplankton at the study site.

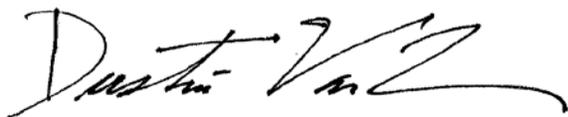
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until the novel and seriously flawed preliminary study by McCauley et al. (2017) can be properly replicated.

Finally, we recommend that NPWS also consider a recent modeling study conducted by the Australian Commonwealth Scientific and Industrial Research Organization (“CSIRO”), which modeled the effect proposed by McCauley et al. (2017) in the context of ocean ecosystem dynamics and zooplankton population dynamics.⁴ The CSIRO report found that even if the full effect claimed by McCauley et al. (2017) did in fact exist, plankton abundance would not be adversely affected—even in areas of intensive seismic surveys—due to the extensive movements of water masses carrying plankton through survey areas and the rapid reproductive cycle and high reproductive potential characteristics of planktonic organisms.⁵ In other words, the purported findings of McCauley et al. (2017) are of no ecological consequence, given the life history parameters of plankton.

Thank you for considering this information. Should you have any questions, please do not hesitate to contact Dustin Van Liew (dustin.vanliew@iagc.org).

Sincerely,



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International Association of Geophysical Contractors
Vice President – Regulatory & Governmental Affairs

⁴ Richardson A.J., R.J. Matear, and A. Lenton. 2017. Potential impacts on zooplankton of seismic surveys. CSIRO, Australia. 34 pp.
<https://publications.csiro.au/rpr/download?pid=csiro:EP175084&dsid=DS1>.

⁵ Planktonic species are considered highly “r” selected, with high rates of density dependent and density independent mortality, countered by high replacement capacity.

REFERENCES

- Booman, C. *et al.* 1996. Effekter av luftkanonskyting på egg, larver og yngel. Undersøkelser ved Havforskningsinstituttet og Zoologisk laboratorium [Effects of airgun pulses on eggs, larvae and fry]. *Fisken og Havet* 1996:3. Norwegian with English Summary.
- Dalen, J. and G.M. Knutsen. 1987. Scaring effects in fish and harmful effects on eggs, larvae and fry by offshore seismic explorations. In: Merklinger, H.M., ed. Progress in underwater acoustics. London: Plenum Press. Pp. 93-102.
- Day, R. D. *et al.* 2016. Assessing the Impact of Marine Seismic Surveys on Southeast Australian Scallop and Lobster Fisheries Final Report 2012-008-DLD (FRDC, 2016).
<http://frdc.com.au/research/final-reports/Pages/2012-008-DLD.aspx>.
- Kostyuchenko, L. P. 1971. Effects of elastic waves generated in marine seismic prospecting on fish eggs in the Black Sea. *Hydrobiol. J.* 9, 45-48.
- McCauley, R. D. in Environmental Implications of Offshore Oil and Gas Development in Australia: The Findings of an Independent Scientific Review (eds. Swan, J. M., Neff, J. M. & Young P. C.) 19–122 (APPEA, 1994).
- McCauley, R.D. *et al.* 2008. Impacts of seismic survey pass-bys on fish and zooplankton, Scott Reef Lagoon, Western Australia: Full report of Curtin University findings. Center for Marine Science and Technology, Curtin University, Perth WA. 92 pp. CMST Report 2008-32.
- Nedelec, S.L. *et al.* 2014. Anthropogenic noise playback impairs embryonic development and increases mortality in a marine invertebrate. *Nature Communications: SCIENTIFIC REPORTS* | 4 : 5891 | DOI: 10.1038/srep05891. www.nature.com/scientificreports/.
- Payne, J. F. 2004. Potential Effect of Seismic Surveys on Fish Eggs, Larvae and Zooplankton. Canadian Science Advisory Secretariat (CSAS/CSSC), Fisheries and Oceans Canada. ISSN 1499-3848. <http://www.dfo-mpo.gc.ca/csas/>. 16 pp.
- Payne, J. F. *et al.* 2009. Potential Effects of Seismic Airgun Discharges on Monkfish Eggs (*Lophius americanus*) and Larvae. Environmental Studies Research Funds Report No.170. July 2009. Science Branch, Fisheries and Oceans Canada, PO Box 5667, St. John's, NL A1C 5X1, Canada. 32+ pp.

- Saetre, R. and Ona, E. 1996. Seismiske undersøkelser og skader på fiskeegg og -larver en vurdering av mulige effekter pa bestandsniv. [Seismic investigations and damages on fish eggs and larvae; an evaluation of possible effects on stock level]. *Fisken og Havet* 1996:1-17, 1-8. Norwegian with English summary.
- Shelley, K. 2011. Reanalysis of “Investigation of the Potential Effects of Low Frequency Sonar Signals on Survival, Development, and Behavior [sic] of Fish Larvae and Juveniles” by Jørgensen et al. Memorandum in support of the decisional record for the Biological Opinion on the US Navy 2010-2015 Keyport Range Complex Extension (FWS #13410-2009-F-0082). Memorandum for the Decisional Record, 31 May 2011. US Fish and Wildlife Service, Lacey WA. 4 pp.
- Stanley, J.A. *et al.* 2011. Behavioural Response Thresholds in New Zealand Crab Megalopae to Ambient Underwater Sound. PLoS ONE 6(12): e28572.
doi:10.1371/journal.pone.0028572.

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ATTACHMENT

Reviewers 1 & 2

APPENDIX: Detailed review of McCauley Paper

This study was conducted in shallow water (34-36m) over two days during March 2015. Duplicate vertical bongo net tows (0.75 m dia, 200 μ m mesh) were conducted from the seabed to the surface at three distances (0, 250, 800m) from the airgun deployment site. These produced n=12 samples per day. Zooplankton taxonomic composition and abundance were determined from analysis of the net samples. A neutral red stain was used on the first two cod ends from each site to assess the presence of dead copepods. The methodology followed Elliot and Tang (2009). A down-looking echosounder (Simrad EK60 120 kHz single-beam) was used to collect pre-airgun (~3h) and post-airgun (~1.5h) transects.

The study reported changes in the abundances of zooplankton prior to, and following, airgun deployments, on both days. They showed a similar taxonomic composition during control (pre-) tows on each day. Acoustic backscatter was higher on day 1 (pre-) than on day 2 (pre-). Fish abundances were similar on both days prior to airgun deployments. The pre-/post- comparisons suggested declines in 58% of zooplankton taxa after airgun exposure. This analysis was based on all ranges (pooled over 0, 250, 800 m ranges). They did assess the range at which reductions were not significant (for copepods and cladoerans, which amounted to 86% of all taxa), and these were 973 – 1119 m. Vital staining indicated more dead animals of all taxa with 2 – 3 x more dead zooplankton present after airgun deployment compared to controls at all ranges. Small copepod taxa (*Acartia tranteri*, *Oithona* spp.) dominated the dead category. There was a non-significant trend of decreasing ratios of dead/total copepods. All post-deployment euphausiids were reported to be dead.

The authors hypothesize that the impact of the airguns was to shake and thereby damage sensory hairs and tissues on zooplankton rendering them dead, or disabled and impaired in sensory ability. This would make them more vulnerable to predation and unable to maintain normal posture and orientation. The hole observed in the echogram was suggested to be a consequence of changes in the orientation of individuals towards a more random distribution relative to the echosounder.

There was no change in the abundance of fish targets pre- and post-deployment but more fish were swimming higher in the water column on day 2 prior to deployment. Juvenile fish sampled by the net were more abundant at 0 and 250 m range prior to deployment and more abundant post deployment at 800 m on day 1. No juvenile fish were collected on day 1 prior to, or after the deployment.

Wavelengths of sound used vs size of zooplankton

Sizes of animals are not provided; however, a 120 kHz echosounder would be able to detect a target with a minimum size of about 0.01 m (= 10 mm). Given that the majority of zooplankton in their samples were smaller than this size, the use of a higher frequency is likely warranted.

Gear used

The use of each net in a single bongo net tow as a true replicate is questionable. Typically one, or the other net is selected for quantitative analysis. Using both nets as replicates with the same power as from individual tows is suggestive of pseudoreplication. Consequently, their statistical power was really based on $n=6$ rather than $n=12$.

Why weren't adult euphausiids detected?

The absence of adult euphausiids (*Nyctiphanes australis*) in their samples can be explained by two potential factors: (1) only larvae were present at the time; or (2) adults were present but they avoided the bongo nets. The authors speculated that the absence of adults was due to their low (0.25 m s^{-1}) tow speeds. It is possible that the population was dominated by juveniles at the time of sampling. Young et al. (1993) sampled *N. australis* off eastern Tasmania and found that the population was dominated by juveniles though the proportion of adults increased during the austral spring and autumn (March – May). McCauley et al. (2017) sampled off southern Tasmania during Autumn so there was temporal match, though not a spatial one. Rich and Hosie (1982) found adult *N. australis* (defined as >11 mm TL) present in the waters off south-eastern Tasmania during March, though the largest adults present at that time (16 mm) were smaller than during other months (up to 20 mm).

Avoidance of the bongo net is a potential explanation for the absence of adults in the samples. Ritz and Hosie (1982) found that an unencased, high-speed plankton net with $250\ \mu\text{m}$ mesh, was effective at sampling all stages of *N. australis* (except the smallest) in waters off south-east Tasmania. However, if the airgun discharge resulted in mortality or sublethal incapacitation of adult euphausiids, then adults should have been present in the post-discharge plankton samples.

Which taxa were affected?

If airgun discharge resulted in mortality or incapacitation of zooplankton, then one would expect to have reduced avoidance of the net following deployment and a hypothesized consequence would be that abundances would be similar to, or higher in post-deployment samples relative to controls within either day. When the three most abundant groups of zooplankton are considered, this hypothesis is not supported except in one comparison.

Copepods (All Taxa)

	0 m Range		250 m Range		800 m Range	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Day 1	1484	476	1240	643	1826	715
Day 2	185	167	120	92	44	235

This hypothesis was supported by only the day 2 samples at 800m range.

Cladocerans (All Taxa)

	0 m Range		250 m Range		800 m Range	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Day 1	245	186	328	164	385	126
Day 2	42	12	20	11	11	41

This hypothesis was supported by only the day 2 samples at 800m range.

Euphausiids (All stages)

	0 m Range		250 m Range		800 m Range	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Day 1	68	21	105	17	83	28
Day 2	15	5	14	0	3	22

This hypothesis was supported by only the day 2 samples at 800m range.

Airguns should not have had an impact on the sampled abundances of taxa that were not mobile or possessed limited mobility. Eggs and egg masses, appendicularia, and the dinoflagellate *Noctiluca scintilans* fall into this category. If similar water masses were sampled pre- and post-deployment, then the abundances should be similar in corresponding net tows.

Appendicularia

	0 m Range		250 m Range		800 m Range	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Day 1	72	43	99	24	177	53
Day 2	19	7	16	6	5	27

This hypothesis is not supported by any comparisons.

Noctiluca scintilans

	0 m Range		250 m Range		800 m Range	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Day 1	0	1	0	5	0	0
Day 2	1	0	0	0	0	0

This taxon was present in very low numbers.

Fish eggs and Egg Masses

	0 m Range		250 m Range		800 m Range	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
Day 1	6	3	33	10	24	15
Day 2	9	4	8	3	8	17

This hypothesis is not supported by any comparisons.

These comparisons suggest the possibility that the differences in abundance measured at each range on each day were due to factors other than the airguns. If the airguns had a direct impact on the zooplankton assemblage, then the mobile taxa should have been as, or more susceptible to capture by the net after airgun discharge. The generally consistent lower abundances immediately following the discharge either indicate that a different water mass was sampled post deployment in which case patchiness would have been responsible for the lower post-deployment abundances, or the impacted animals were rapidly removed from the water column either by predation, sinking, or a combination of the two processes. The time interval between airgun discharge and the collection of plankton net samples is not provided in either the manuscript or the supplementary material so it is not possible to determine how far apart the events were. A drifter was used to estimate where the deployment site was relative to the plankton net tows; however, the assumption is that the water mass moved as a uniform vertical mass without shear and the drifter (which was drogued at 5m depth) tracked the water mass.

Use of vital stains to evaluate mortality

The methodology described by Elliot and Tang (2009) requires several safeguards to minimize collection and post-collection mortality. These include limiting the tow velocity to $<1 \text{ m s}^{-1}$; restricting the tow distance based on a formula that includes the zooplankton abundance; and not rinsing down the net. McCauley et al (2017) kept their tows to 0.25 m s^{-1} . Their tow distances (estimated from Supplementary Table 6 by dividing volumes by πr^2 (0.4418 m^2) were: day 1 control = 55.2m ; day 1 active = 52.7m; day 2 control = 54.3m; day 2 active = 43.7m. These numbers do not provide any information on each of the three range samples (0 m, 250 m, 800m) so it is not possible to estimate the tow distances corresponding to the 12 tows listed in Supplementary Table 1. Equation (1) from Elliot and Tang (2009) estimates the recommended tow distance (m) as $38200/\text{in situ zooplankton concentration (n m}^{-3})$. The basis of this formula appears to be that the higher the abundances present, the shorter the tow distance in order to minimize mortality due to crowding in the cod end. Using this formula and the total zooplankton abundances provided in Suppl. Table 1 we get the following recommended tow distances.

Range	Day 1 Pre	Day 1 Post	Day 2 Pre	Day 2 Post
0 m	19.7	50.4	138.7	395.8
250 m	20.1	42.3	208.8	304.0
800 m	14.6	39.0	520.5	107.3

Based on these numbers, it appears that the tow distances were all within the recommended maxima except on Day 1 prior to the airgun discharges. Assuming that the pre-discharge samples collected on day 1 had resulted in elevated collection mortality due to excessive tow durations, the differences in mortality between pre- and post-sampling on day 1 are likely even more pronounced. No information on how the nets were rinsed is provided, nor is there any information on whether the nets were thoroughly rinsed between samples to purge dead copepods as is recommended in Elliot and Tang (2009).

References

- Elliot, D.T. and K.W. Tang. 2009. Simple staining method for differentiating live and dead marine zooplankton in field samples. *Limnol. Oceanogr. Meth.* 7, 585–594.
- McCauley, R.D., R.D. Day, K.M. Swadling, Q.P. Fitzgibbon, R.A. Watson, and J.M. Semmens (2017), Widely used marine seismic survey air gun operations negative impact zooplankton. *Nature Ecology and Evolution* 1, 1-8.
- Ritz, D.A. and G.W. Hosie. 1982. Production of the Euphausiid *Nyctiphanes australis* in Storm Bay, South-Eastern Tasmania. *Marine Biology*, 68: 103 – 108.
- Young, J.W., A.R. Jordan, C. Bobbi, R.E. Johannes, K. Haskard, and G. Pullen. 1993. Seasonal and interannual variability in krill (*Nyctiphanes australis*) stocks and their relationship to the fishery for jack mackerel (*Trachurus declivis*) off eastern Tasmania, Australia. *Marine Biology*, 115: 9 – 18.

Reviewer 3

McCauley et al. 2017 - Review and recommendations for improvements.

Summary:

This paper presented a compelling argument that seismic airgun surveys directly impact zooplankton survival to a distance of at least 800 m. The study was not particularly well designed to address the effects of airgun surveys, but the authors made the best of the data they had available. The biggest strength of the dataset was the neutral red dye evidence of significant mortality following the airgun experiment. The biggest flaw was the incomplete assessment of water mass movements before and after the experiments, which complicated interpretation of the data. Their method used to locate the sonar pings which best represented water parcels sampled at different distances from airgun fire, adjusted in time and space for drift, was as well done as could be given the limited water velocity data collected, but could be dramatically improved. Additional issues with the methodology (e.g., a mismatch between zooplankton size ranges ensonified using 120 kHz sonar versus those captured using a vertical net tow protocol) and some choices made during data analysis muddle the results.

Specific issues:

Sonar: The “hole” that developed in the sonar backscattering is very good evidence that the airguns elicited a large response by the zooplankton, but it is not sufficient to show mortality. The main issue is that the question “where did the bodies go?” was not addressed, and indeed, a tight, intensified scattering layer seems to have developed deeper in the water column (Figure 4c,d,g) that the authors did not address. That deeper layer may have resulted from a vertical avoidance response (i.e., swimming down) that is a very common zooplankton response to predation threat. Note that the authors suggest that the “hole” could have been an artifact of the sonar interacting with the swimming angle of the zooplankton. During active vertical migrations, such an effect (an apparent disappearance of zooplankton scattering layers) is a well known artifact of acoustic sampling, but such a behavioral change doesn’t seem sustainable over the period of time shown, and seems especially unlikely in combination with the appearance of the deeper Sv layer that developed. It seems much more likely that the zooplankton exhibited an escape response, swimming down where they then aggregated closer to the bottom. The shift from the near-surface scattering to the deep scattering probably appeared too quickly for it to be caused by sinking of dead individuals (unless they were all large), but is within the swimming speed of the larger fraction of these zooplankton, which swim strongly. It is notable that the

authors focused their analyses only on the small layer of the water column where backscatter decreased, never analyzing integrated water column backscatter.

Zooplankton sampling: The methods to quantify the zooplankton were mis-matched in this study. The 200 um mesh vertical net tows will only capture small organisms that cannot avoid nets (especially when lifted slowly as per the Neutral Red Dye protocol), whereas the 120 kHz sonar will mostly backscatter off larger taxa (except when small taxa are very densely aggregated). That leads to a mis-match between conclusions that can be drawn from the sonar and those that can be drawn from the net tows. Too few net tows were conducted to provide convincing evidence of a change in abundances, especially since the flow meters didn't work well, so the volumes filtered were very questionable. Furthermore, within 1.5 hours, there should be very little change in whole-water-column abundances if direct mortality occurred, as the sinking bodies would still be sampled by the nets and acoustics – it would take a few hours for something copepod-size to settle out completely. In combination with fairly poor water-mass tracking, the abundance changes described were fairly weak evidence of zooplankton mortality.

Water mass advection: There is decent evidence that the airgun experiments were not conducted in very homogeneous water, making the before-versus-after comparisons difficult in the absence of very good water parcel tracking, and especially casting doubt on the Day 1 versus Day 2 comparison. First, the two CTD casts taken at approximately the same location (on Day 2) showed different salinity profiles, indicating the water moving past that location differed from before to after the survey. The sonar surveys showed quite a bit of biological pattern (not surprising). Furthermore, the control:exposed abundances (Table S1) showed an *increase* in many taxa at 1200 m (nominally the 800 m distance) on Day 2 after exposure relative to the average pre-exposure abundances – the authors argue those data are evidence that the airguns didn't affect zooplankton that far afield, but the data also demonstrate the biological patchiness that make time/space comparisons difficult in any non-homogeneous ocean.

The authors suggest that Day 1 and Day 2 can be compared because they sampled at about the same phase of an oscillating tide. However, they describe significant wind stress (12-18 kts on Day 2) and don't describe the tidally-averaged circulation of the region. There is no reason to think that the water mass present at that location on Day 1 was the same as on Day 2.