



The copepod *Acartia* sp. is more sensitive to a rapid pressure drop associated with seismic airguns than *Calanus* sp.

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ABSTRACT: Airguns used in seismic surveys release high-pressure air, generating sound waves that may have adverse effects on marine life. However, knowledge of how seismic exposure impacts zooplankton is limited. One key characteristic of seismic signals that could potentially cause damage is a rapid pressure drop. In this study, the rapid pressure drop (~2 bar) was re-created in the laboratory using a pressure tube. To determine the range at which this drop occurs, the sound field around a seismic airgun array was modeled. The effects of this pressure drop on mortality and swimming behavior were tested in 2 common copepods, *Acartia* sp. and *Calanus* sp., both immediately and 5 h after treatment. Pressure-exposed *Acartia* sp. showed higher mortality rates (0 h: 5.6%; 5 h: 10%) compared to the controls, while mortality in *Calanus* sp. only increased after 5 h (3.3%). The swimming speed of pressure-exposed *Acartia* sp. (0 h: 0.49 mm s⁻¹; 5 h: 0.52 mm s⁻¹) was lower than in the control treatment, whereas the swimming speed in pressure-exposed *Calanus* sp. (2.64 mm s⁻¹) only differed immediately after treatment. This study demonstrates that a rapid pressure drop can negatively affect zooplankton mortality and behavior at close range. The results also show that *Acartia* sp. is more sensitive to this pressure drop than *Calanus* sp., suggesting potential species-specific impacts from seismic exposure. Identifying the sound characteristics that can be harmful to zooplankton allows for a more accurate assessment of the most affected species and the range at which impacts can occur.

KEY WORDS: Seismic surveys · Anthropogenic noise · Impulsive underwater noise · Zooplankton · Mortality · Mobility · Hydrostatic pressure

1. INTRODUCTION

An increase in human activities in marine environments, including ship traffic, pile driving, dredging, and seismic surveys, has resulted in the recognition of noise pollution in the oceans (Williams et al. 2015, Duarte et al. 2021). Anthropogenic underwater noise, both impulsive and continuous, may have adverse effects on marine life (Williams et al. 2015, Merchant et al. 2022). However, limited information is available regarding the impact of seismic surveys on marine organisms occupying lower trophic levels, such as zooplankton and other invertebrates (Carroll et al.

2017, Solé et al. 2023, Vereide & Kühn 2023). Airguns are the most common and efficient type of marine seismic source in the search for oil and gas deposits. They transmit loud low-frequency (< 100 Hz) sound waves that propagate thousands of meters down in the sediments under the seabed. A single seismic survey may cover an area of more than 2000 km², shooting approximately every 10 s over weeks or months (Weilgart 2013, Slabbekoorn et al. 2019). Seismic exposure can affect marine fauna and cause direct and indirect damage, such as changes in predator–prey interactions or species composition (Todd et al. 2015, Kavanagh et al. 2019, Slabbekoorn et al. 2019).

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Furthermore, seismic airguns impact animals in different ways, e.g. by overlapping the frequencies used in the communication of whales (Kavanagh et al. 2019) or by affecting the behavior of fish through the detection of particle motion or sound pressure (Slabbekoorn et al. 2019). Most of what is known about the effects of seismic exposure on marine life comes from studies on mammals and fish (Gordon et al. 2003, Popper & Hawkins 2016, 2019).

Only a few studies have investigated the effects of seismic activities on zooplankton (Solé et al. 2023), despite their crucial role in marine ecosystems (Pinti et al. 2023a). These studies have reported a range of impacts, including severe physical damage and mortality as well as no significant effects (Carroll et al. 2017, Vereide & Kühn 2023). For example, Fields et al. (2019) reported low immediate mortality (~10% in the exposed vs. ~2% in the control) in *Calanus finmarchicus* after exposure to 2 small airguns at a short distance (<5 m). Similarly, Vereide et al. (2023) showed a small increase in immediate mortality (~14% in the exposed vs. ~4% in the control) in *Acartia tonsa* nauplii when exposed to 2.5 h of seismic blasting at different distances (50 m to 1.2 km). Pearson et al. (1994) observed no effects on mortality or development in crab larvae (*Cancer magister*) after seismic exposure. The low levels of mortality in these 3 studies contrast with those of McCauley et al. (2017), who reported a substantial increase in mortality (~45% in the exposed vs. ~20% in the control) in natural zooplankton communities after seismic exposure up to a maximum sampling distance of >1 km from an airgun source. These variations may be caused by species-specific differences in sensitivity or by differences in sound exposures, such as sound levels, sound sources, or exposure time. In this study, we tested the effect of the same exposure in 2 different zooplankton species.

Zooplankton exhibit diversity in both their physical characteristics and functions (Lindeque et al. 2013, Deagle et al. 2018). They vary in size (Evans et al. 2020, Brandão et al. 2021) and display distinct behaviors (Kiørboe et al. 2010a, Almeda et al. 2017). Consequently, the impact of exposure may differ among zooplankton species and taxa, depending on factors such as their size, physiology, or behavior. Among the studies investigating the effects of seismic exposure on copepods (McCauley et al. 2017, Fields et al. 2019, Vereide et al. 2023), the mortality and growth of copepods are negatively affected, albeit to varying degrees. However, these studies have investigated the effects in different species. For example, large copepods have shown no behavioral responses and experienced lim-

ited but adverse effects on mortality (Fields et al. 2019), whereas zooplankton communities dominated by small copepods have been reported to be more affected (McCauley et al. 2017). These variations may be caused by species-specific differences in sensitivity. Therefore, to test the differences between copepod species, we used 2 genera of zooplankton that are common in both coastal and open-ocean marine environments: *Acartia* and *Calanus*.

Aside from mortality, our understanding of the impact of seismic surveys on zooplankton behavior is even more limited. Sound exposure can cause physical harm to marine animals, including zooplankton, which may result in decreased sensitivity or ability to move (Solé et al. 2021). Fields et al. (2019) found no changes in the escape behavior of *C. finmarchicus* following airgun exposure. However, they only examined escape behavior and not overall activity. Copepods are dependent on mechanoreceptive setae for sensing and reacting to external stimuli (Fields et al. 2002, Solé et al. 2021). Therefore, alterations in the behavior of copepods, such as changes in swimming activity, could potentially indicate sub-lethal physical damage. The swimming behavior of zooplankton can be affected by external factors like turbulence, which may hinder their ability to evade predators (Visser et al. 2008). Consequently, changes in mobility, in addition to being an indication of sub-lethal damage, could suggest reduced survival ability in the field (Buskey et al. 2002). Therefore, our study tested the effects of exposure on swimming activity in zooplankton to assess any potential adverse effects on their condition.

When an airgun is fired, high-pressure compressed air (typically 137 bar) is released (Caldwell & Dragoset 2000) into the surrounding water, forming a rapidly expanding bubble. The resulting sound wave, or acoustic signal, consists of an initial high-amplitude pressure pulse, followed by decaying pulses formed by oscillations of the resulting air bubble (Dragoset 2000). To increase the source energy and focus more energy downward, several individual airguns (typically 18–48) are arranged in an airgun array. Airguns are usually deployed at depths of 5–15 m (Prior et al. 2021), and the surface reflection of the signal is added to the transmitted pulse with a short delay. The reflected surface signal has an opposite phase, causing the positive pressure peak to be reflected as a negative pressure peak. Therefore, positive pressure is followed by negative pressure, resulting in a fast hydrostatic pressure drop (McCauley et al. 2021). This pressure drop is typical for impulsive signals and much larger and steeper than the pressure

fluctuations observed in continuous sounds like boat noise. For both seismic exposures and detonations, this phenomenon can be fatal for marine animals near the source, particularly if they have air inclusions. Even fish without swim bladders have been observed to die near detonations, likely due to the oscillations of microbubbles in their tissues (Goertner et al. 1994). Sound waves generated by airguns can propagate over 1000 km from the source (Thode et al. 2010), but their amplitude decreases rapidly in close proximity to the source and less rapidly as the distance from the source increases (Caldwell & Dragoset 2000). Thus, to assess at what distances potential effects may occur, we modeled at what range from an airgun array this pressure drop will occur.

Copepods are regularly exposed to hydrostatic pressure changes as they undergo diel and seasonal vertical migration, since pressure changes at 0.1 bar per meter depth (Hays et al. 1994, Bandara et al. 2021, Pinti et al. 2023b). For example, *C. finmarchicus* can migrate to depths between several hundred and >2000 m, which would result in a pressure change of up to 200 bar (Kvile et al. 2022). The magnitude of the pressure drop caused by commonly used marine seismic sources (Caldwell & Dragoset 2000) is not nearly as intense. For example, a seismic airgun array with 2730 in³ (~44 737 cm³) volume and 137 bar firing pressure would result in an approximate 9 bar drop in the hydrostatic pressure immediately adjacent to the source, which decreases rapidly with distance (Khodabandeloo et al. 2017). However, with a common ascent swimming speed of <6 mm s⁻¹ (Berge et al. 2014), these natural changes occur relatively slowly (6×10^{-7} bar ms⁻¹) compared to the pressure drop that animals are exposed to close to a seismic airgun (~0.4 bar ms⁻¹). Therefore, the rapid pressure drop that occurs close to seismic airguns may cause damage and is likely one of the characteristics underlying the negative effects of an exposure to sound from seismic airguns reported in the literature. Here we simulated this hydrostatic pressure drop in isolation, excluding other aspects of sound exposure, such as particle motion.

In this study, we tested the effects of a rapid pressure drop associated with seismic airguns on the mortality and swimming behavior of 2 genera of copepods, *Acartia* and *Calanus*. Based on the previously observed differences, we hypothesized that the negative effects would be stronger in *Acartia* sp. than in *Calanus* sp. In addition, to assess whether damage from seismic surveys could lead to population-level effects, we modeled at what range this pressure drop can occur in the sound field around a seismic airgun array.

2. MATERIALS AND METHODS

All experiments were conducted at the Institute of Marine Research, Austevoll Research Station (60° 5' 9.02" N, 5° 15' 41.94" E), between 23 August and 9 September 2022 (Table 1). During the experiments, *Acartia* sp. and *Calanus* sp. were sampled in the field (60° 5' 9.51" N, 5° 15' 22.49" E; 60° 5' 18.09" N, 5° 16' 0.91" E) and exposed to either a pressure drop or a control treatment, following measurements of swimming behavior and mortality immediately and 5 h after treatment (see Section 2.5).

2.1. Experimental animals

The study focuses on *Calanus finmarchicus* (Gunnerus, 1770) and *Acartia tonsa* Dana, 1849, as they constituted the majority of the genera *Calanus* and *Acartia*, respectively, which were sampled at the time of the study in Austevoll.

C. finmarchicus is a calanoid epipelagic copepod that is most commonly found in the Norwegian Sea and North Sea. In these areas, *C. finmarchicus* may account for more than 80% of mesozooplankton biomass (Aarflot et al. 2018). The species is considered a large copepod (2–4 mm) that accumulates large lipid stores (up to 31% of the total dry weight) (Lee et al. 2006), converting carbon from phyto- and microzooplankton to accessible energy (Skottene et al. 2020).

A. tonsa is also a calanoid epipelagic copepod distributed throughout the oceans of the world but is commonly found in coastal and estuarine areas (Cervetto et al. 1995). In Norwegian coastal waters, *A. tonsa* is often one of the dominant species of smaller copepods throughout summer and spring and serves as an important food source for many fish species (Sullivan et al. 2007). The body length of adult *A. tonsa* ranges from 0.5 to 1.5 mm, and the species contains low lipid reservoirs compared with *C. finmarchicus* (Lee et al. 2006).

2.2. Sampling of animals

Acartia sp. was sampled using a WP2 plankton net (mesh size 180 µm) (Table 1). The plankton net was towed behind a small boat at approximately 1 m s⁻¹ at a depth of 10 m. Two net hauls were collected on each sampling day (Table 1). *Calanus* sp. was sampled using a light trap at a depth of 20 m. The light trap was deployed at night and returned the following morning (Table 1). All sampling and treatment times can

Table 1. Overview of species, sampling, environmental conditions, and treatments. Each set consisted of a pressure exposure and a control. Dates are given as d/mo/yr

Species	Sampling gear	Sampling depth (m)	Sampling date	<i>In situ</i> temp. (°C)	<i>In situ</i> salinity (psu)	Treatment date	Sets (pressure exposure + control)
<i>Acartia</i> sp.	WP2 plankton net (180 µm)	10	23/08/22	17.2	31.1	24/08/22	1 and 2
			25/08/22	17.0	31.1	26/08/22	3 and 4
			25/08/22	17.0	31.1	26/08/22	5 and 6
<i>Calanus</i> sp.	Light trap (200 µm)	20	06/09/22	16.0	31.7	07/09/22	1 and 2
			08/09/22	16.0	31.7	09/09/22	3 and 4
			08/09/22	16.0	31.7	09/09/22	5 and 6

be found in Table 1. Both of the sampling locations were in close proximity to the laboratory. After sampling, the nets and cod ends were rinsed, and all animals were transferred to large buckets filled with seawater. Subsequently, the buckets were carried to the laboratory, where they were immediately placed in a climate-controlled room at a constant temperature, adjusted to the *in situ* temperature at the current sampling times (Table 1). Slow aeration was then applied to the buckets. The light:dark cycle was 12:12 h, and no food was provided.

2.3. Pressure tube and pressure measurements

The pressure tube consisted of a polyvinyl chloride (PVC) tube (external diameter: 38 mm; length: 260 mm). Valves (25 mm; FIP Easyfit PVC-U Ball) were attached to both ends (Fig. 1). One of these valves was used to introduce and retrieve the water and animals, the other was used to release the pressure.

A 4.8 kHz digital dynamic pressure sensor (Applied Measurements Limited, USB Pa-USB-FQ) was attached to the tube wall to measure the pressure inside

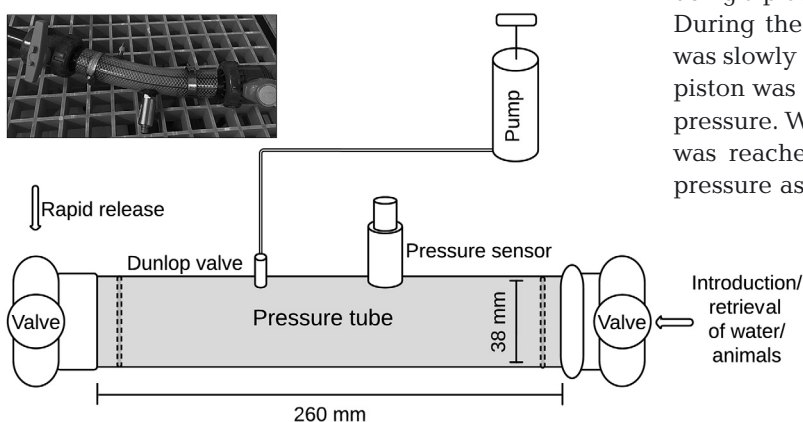


Fig. 1. Setup of the pressure tube with notations and placements of its components. Inset: pressure tube

the chamber (Fig. 1). The pressure sensor was connected to a PC via a USB cable, from where the pressure was observed, using the FSU Toolkit software (Mantracourt, version 01.03). A Dunlop valve was also attached to the tube, to which a pump with a hand-operated piston was connected (maximum pressure 160 psi/11 bar, height 67 cm).

2.4. Experimental setup

Six sets of each treatment (control, pressure exposure) were conducted for both species (Fig. 2; Table 1). For each treatment, 30 individuals were counted and picked using a Leica stereomicroscope (Leica Microsystems, Stereozoom S9i) and then transferred to an evaporating glass dish filled with filtered seawater. The same stereoscope was used for all measurements. Second, the pressure tube and pump were filled with filtered seawater at the *in situ* temperature, and all animals were carefully poured from the glass dish into the tube while avoiding the introduction of air bubbles. Any remaining air bubbles were removed by carefully sweeping the inner walls of the tube using a plastic strip. Thereafter, the valve was closed. During the pressure exposure treatment, the piston was slowly launched to push water into the tube. The piston was launched only once to reach the targeted pressure. When the target absolute pressure of ~3 bar was reached, the valve was opened to release the pressure as quickly as possible, resulting in a ~2 bar pressure drop. The absolute pressure indicates a measure using absolute zero as a reference point. Therefore, when the pressure is released by ~2 bar, the pressure returns to the atmospheric pressure (~1 bar, 1 atm = 1.01325 bar). After each treatment, the animals were carefully poured from the tube into a container for further measurements.

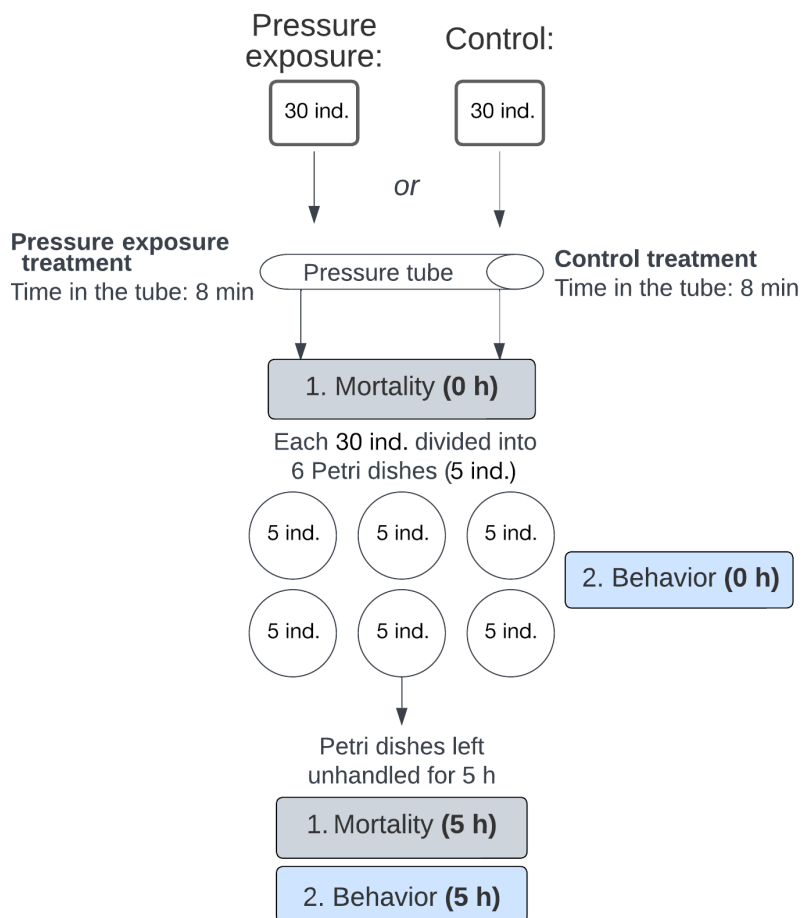


Fig. 2. Experimental setup. The setup was identical for *Acartia* sp. and *Calanus* sp. (1) Mortality of the copepods of each treatment (pressure exposure and control) was measured immediately after treatment, with a total of 6 sets (6× pressure exposure, 6× control). After dividing 30 individuals into 6 Petri dishes, the animals were recorded, and (2) the swimming behavior was measured. The same measurements were conducted after 5 h

The tube was rinsed several times to ensure that all individuals had been removed. The full exposure treatment lasted 8 min from introduction to removal of the animals. Therefore, the animals were kept in the tube for 8 min during the control treatment, and treated identically, although without changing the pressure. The order of the control and the corresponding exposure treatment were randomized.

2.5. Mortality and activity measurements

Immediate mortality was investigated within 10 min of treatment using a stereoscope by carefully stimulating the animals using a plastic pipette. Animals that did not exhibit any response within 10 s of stimulation were considered dead. After measurements, the 30 in-

dividuals from each treatment were haphazardly divided into 6 plastic Petri dishes (diameter 95 mm, height 15 mm) filled with filtered seawater at *in situ* temperature. Both dead and live animals were included in the recordings to confirm that the animals were dead and not stunned by the pressure exposure. Thus, there were 5 individuals per dish (Fig. 2). To record activity, these 6 Petri dishes were placed beneath 3 cameras (SONY HDR-GW55VE, HDR-CX280E). After an acclimatization period of 30 s, the dishes were recorded for 4 min (van Duren & Videler 1995). The Petri dishes were then left unhandled for 5 h in the same climate room. After 5 h, mortality was measured again in the same manner as previously described, followed by the same recording procedure for activity. Finally, after all measurements were taken, pictures of all individuals were taken using an AirLab 2.0 Leica Microsystems equipped with a Leica CLS150 LED light. From the pictures, the prosome length of all individuals was measured using the software ImageJ (version 1.53e) (Schneider et al. 2012).

All video recordings of copepod swimming activity were analyzed using the annotation tool software Kinovea 0.9.5 (Charmant 2021). The path of each copepod was tracked during the 4 min of recording, from which parameters such as distance, speed, and coordinates could be exported. In addition, for each dish, it was noted whether the individual was alive or dead; the latter was excluded from behavioral analyses. Furthermore, as it was not realistically possible to identify and track the same individual within each dish immediately and after 5 h, we used the mean speed of the live individuals in each Petri dish.

2.6. Modeling approach: hydrostatic pressure variations around a seismic airgun array

To understand how a seismic airgun array impacts the hydrostatic pressure in its vicinity, the pressure field at time t and location (x, y, z) was modeled using notional source signatures (Ziolkowski et al. 1982, Khodabandeloo 2018) as:

$$p(t, x, y, z) = 10^5 + \rho z g + \sum_{i=1}^N \frac{1}{r_i} p_{ni} \left(t - \frac{r_i}{c} \right) + \sum_{i=1}^N \frac{R}{r_{gi}} p_{ni} \left(t - \frac{r_{gi}}{c} \right) \quad (1)$$

where N is the number of airguns in the array, p_{ni} is the notional source signature of the i^{th} airgun in the array. The distances between the point (x, y, z) and the i^{th} airgun, as well as its reflected acoustic wave from the sea surface (referred to as ghost), are shown by r_i and r_{gi} , respectively. Depth is shown by z . The speed of sound is represented by c , its density by ρ , and $R = -1$ is the reflection coefficient from the sea surface. The notional source signatures were modeled using the seismic air gun modeling package NUCLEUSTM, a product of Petroleum Geo-Services. The airgun array consisted of 30 airguns (total 2730 in³) arranged in 3 sub-arrays (see Khodabandeloo et al. 2017). The hydrostatic pres-

sure changes (maximum minus minimum) at different locations around the array caused by the acoustic pressure propagation from the airgun were modeled using Eq. (1) and are plotted in Fig. 3.

2.7. Theoretical modeling of cavitation and bubble-dynamics

Acoustic waves generated by seismic airguns have the potential to cause cavitation by reducing the pressure below the vapor pressure of water (Mellen 1954, Plesset 1970). Cavitation can occur at these locations, which causes extreme expansion and sudden collapse at cavitation nuclei sites (e.g. microbubbles) (Mellen 1954, Ceccio & Brennen 1991). Seawater microbubbles, which act as nucleation sites, have

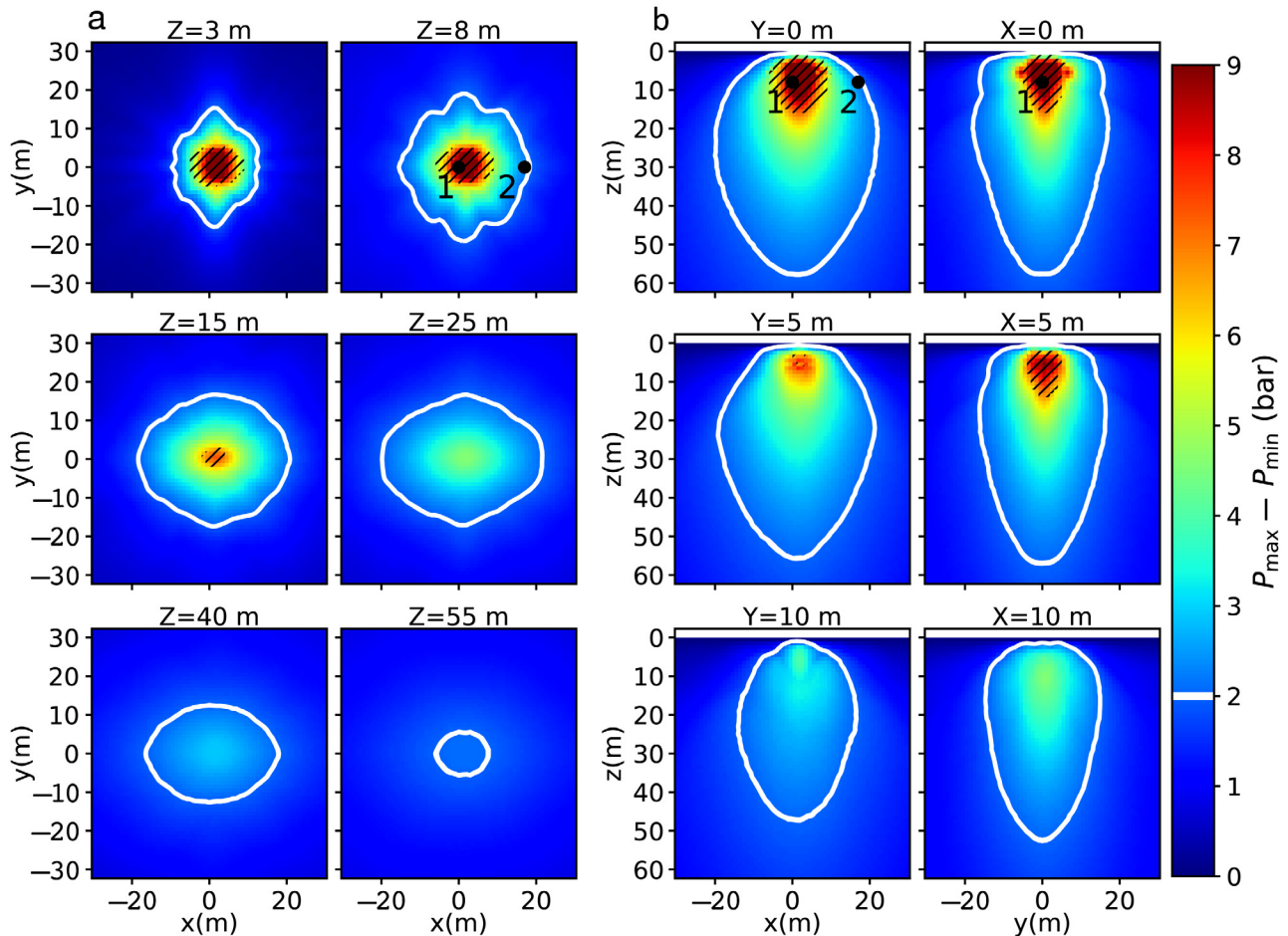


Fig. 3. Pressure drop (difference between the maximum and minimum pressures) at different distances from an airgun array. The modeled airgun was a 2730 in³ (~44 737 cm³) array located at a depth of 5 m. x is the direction the airgun travels (positive x points towards the bow), positive y is the starboard direction, and positive z is the water depth measured from the water surface. (a) Top view of the pressure drop at different depths. (b) Side view of the pressure drop at different x, z and y, z planes with offsets (0, 5, and 10 m). The white solid line indicates 2 bar, which is equivalent to the pressure drop in the pressure tube experiment. The hatched area refers to the area in which the absolute pressure drops below the water vapor pressure and cavitation could occur. The response of a microbubble at locations '1' and '2' (marked by black dots) is plotted in Fig. 4

radii between 1 and 100 μm (Ceccio & Brennen 1991). Cavitation can cause severe harm to nearby organisms, and snapping shrimp utilize this phenomenon to stun or kill their prey (Versluis et al. 2000). When cavitation occurs, a 20 μm bubble can grow up to approximately 11 mm (Fig. 4a), and its subsequent collapse generates an intense acoustic wave (Versluis et al. 2000, Khodabandelloo et al. 2017). To test whether cavitation could have occurred in our setup, we modeled the response of a microbubble subjected to pressure variations around the seismic airgun and pressure tube using equations that govern bubble dynamics (Fig. 4) (Prosperetti & Lezzi 1986, Khodabandelloo et al. 2017).

2.8. Data analyses

All data analyses were implemented using R (version 4.2.2) (R Core Team 2022). For all analyses, a significance threshold of 5% was used. First, to test whether the pressure drop the animals were exposed to differed between species, the pressure measurements from the pressure exposure sets for each species were tested using a paired t -test. Here, the difference in the mean pressure drop between *Acartia* sp. and *Calanus* sp. was tested (Table 2), i.e. if the pressure drop rate was different between copepod groups. The effect of treatment on mortality was tested separately for *Acartia* sp. and *Calanus* sp. immediately and 5 h after treatment. Because there was a total absence of dead individuals in the control treatment group for *Acar-*

tia sp. immediately after treatment, and for *Calanus* sp. both immediately after and after 5 h, and thus a lack of variation within those treatments, it was not possible to apply binomial models to compare mortality between different treatments. Consequently, the impact of treatment on mortality was assessed by conducting Kruskal-Wallis tests separately for each time point after treatment for both groups ($n_{\text{replicate}} = 12$; 6 per treatment) (Table 2).

The effect of treatment on behavior (swimming speed) was first tested in a global model using a generalized linear mixed-effect model (GLMM). In this model, we used the mean speed in each dish as a dependent variable. We included treatment, time after treatment (0 and 5 h), and species as fixed factors, and replicate as random factor. We also included the interactions between time after treatment and species, treatment (pressure exposure vs. control) and species, and treatment and time after treatment as fixed effects. To account for a potential effect of the time the animals spent in the laboratory between sampling and the start of the experiment (Table 1), we added days between sampling and experiment as a covariate (Table 2). Secondly, to test the effect of treatment separately for each time point (0 and 5 h), we used 4 separate models (GLMMs) with the mean speed within each dish as a dependent variable, with treatment (pressure exposure vs. control) as a fixed effect and replicate as a random factor (Table 2; $n_{\text{dish}} = 72$; 36 per treatment; $n_{\text{replicate}} = 12$; 6 per treatment). Because a difference in copepod size could result in a difference in speed unrelated to treatment (Svetlichny et al.

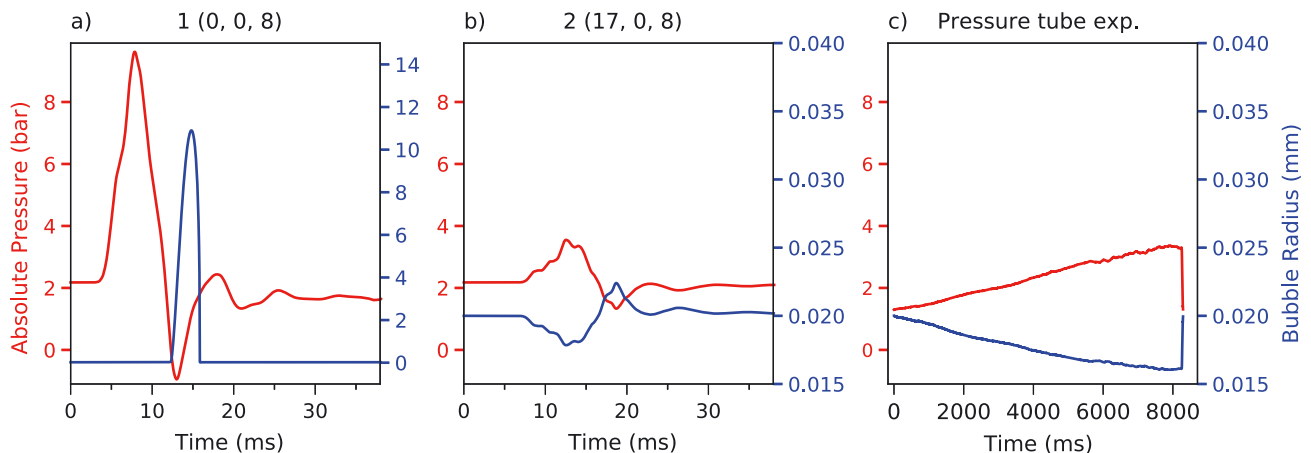


Fig. 4. Modeled response of a microbubble (blue line) to the pressure variations (red line) using bubble dynamics equations. (a,b) A microbubble with radius 20 μm in response to the pressure variations at 2 different locations (marked '1' and '2' in Fig. 3). (c) Response of a 20 μm microbubble to the pressure variations in the pressure tube experiment. The titles illustrate the coordinates of the locations where the pressure is modeled (x [m], the direction the airgun travels; y [m], starboard direction; and z [m], the water depth measured from the water surface)

Table 2. Overview of statistical tests. (a) Paired *t*-test on the effect of species on pressure drop rate. (b) Kruskal-Wallis test on the effect of treatment on the proportion of dead individuals for *Acartia* sp. and *Calanus* sp. after 0 h and 5 h, separately. (c) Generalized linear mixed-effect model (GLMM) (global model) on the effect of treatment, species, time after treatment, as well as the interactions, and days between sampling and experiment (Days) on mean swimming speed for each Petri dish. (d) GLMM (separate models) on the effect of treatment (control, pressure exposure) on mean swimming speed for each Petri dish. Significant values are displayed in **bold**

(a) Pressure drop rate		Effects	95% CI (min./max.)	<i>t</i>	p			
		Pressure drop rate	−0.012/0.009	−0.303	0.774			
(b) Mortality		Group	Effects	df	χ^2	p		
	<i>Acartia</i> 0 h	Pressure	1	7.301	7.0×10^{-3}			
	<i>Acartia</i> 5 h	Pressure	1	9.103	3.0×10^{-3}			
	<i>Calanus</i> 0 h	Pressure	1	3.667	0.056			
	<i>Calanus</i> 5 h	Pressure	1	5.333	0.021			
(c) Swimming activity		Global model		Effects	Estimate	SE	<i>t</i>	p
				(Intercept)	1.172	0.391	2.996	3.0×10^{-3}
				Pressure	1.304	0.319	4.083	< 0.0001
				<i>Calanus</i>	−0.648	0.328	−1.974	0.048
				Time (0 and 5 h)	6.0×10^{-3}	0.083	0.07	0.944
				Days	−0.144	0.244	−0.591	0.555
				Pressure: <i>Calanus</i>	−1.223	0.468	−2.610	0.01
				Time: <i>Calanus</i>	0.063	0.088	0.712	0.476
				Pressure:Time	−0.046	0.052	−0.879	0.379
Separate models		Model	Effects	Estimate	SE	<i>t</i>	p	
	<i>Acartia</i> 0 h	(Intercept)	1065.7	115.8	9.202	< 0.0001		
		Pressure	1505.4	115.4	13.045	< 0.0001		
	<i>Acartia</i> 5 h	(Intercept)	1006.3	154.4	6.519	< 0.0001		
		Pressure	1139.7	243.3	4.684	< 0.0001		
	<i>Calanus</i> 0 h	(Intercept)	303.14	23.92	12.675	< 0.0001		
		Pressure	80.53	34.66	2.323	0.02		
	<i>Calanus</i> 5 h	(Intercept)	373.48	27.77	13.448	< 0.0001		
		Pressure	41.11	38.36	1.071	0.28		

2020), we tested for differences in copepod prosome length between treatments using a *t*-test.

3. RESULTS

3.1. Pressure measurements

The mean \pm SD of the maximum absolute pressure in an exposure was 2.97 ± 0.05 bar for *Calanus* sp. ($n = 6$) and 2.93 ± 0.08 bar for *Acartia* sp. ($n = 6$) (Fig. A1 in the Appendix). The pressure drop of ~ 2 bar had a mean \pm SD drop rate of 0.06 ± 0.01 bar ms^{-1} during the pressure exposure sets of *Calanus* sp. and 0.05 ± 0.01 bar ms^{-1} in *Acartia* sp. There was no significant difference in either the maximum pressure or mean drop rate in pressure between the exposures of the 2 species (Table 2).

3.2. Pressure drop in the experiment vs. that around a seismic airgun array

The hydrostatic pressure drop around a seismic airgun array due to its acoustic wave was modeled using Eq. (1) and is represented spatially in Fig. 3. The airgun array used in the modeling consisted of 3 sub-arrays with a total volume of 2730 in^3 (see Khodabandloo et al. 2017). The rapid pressure drop in the experimental setup was ~ 2 bar.

A hydrostatic pressure drop of 2 bar or more occurred within a horizontal radius of 5 m around the airgun array at a depth of 55 m and 20 m around the airgun array at a depth of 8 m (Fig. 3). Vertically, this area extended to a maximum of 60 m directly below the airgun array (Fig. 3). For the given airgun array, the area in which cavitation may occur, e.g. where the absolute hydrostatic pressure dropped below the

water vapor pressure of 0 bar, extended to a maximum of ~10 m horizontally and ~15 m vertically (Fig. 3). However, it should be noted that the pressure drop in the seismic survey in this area occurred more rapidly than the pressure drop we re-created in the pressure tube (Fig. A1).

To illustrate where cavitation occurs, the response of a microbubble with an equilibrium radius of 20 μm subjected to pressure variations at 2 specific points (labeled '1' and '2' in Fig. 3) was modeled using the bubble dynamics equations (Fig. 4). The pressure tube simulated the hydrostatic pressure drop in the area around the airgun array where cavitation was not expected (Fig. 4b,c). In addition, the modeled response of a similar-sized microbubble subjected to pressure variations within the pressure tube did not indicate cavitation (Fig. 4c).

3.3. Mortality

In *Acartia* sp., the proportion of dead individuals in the pressure exposure treatment was significantly higher than that in the control treatment, both immediately and 5 h after treatment (Table 2). The mean \pm SD mortality in the pressure-exposed *Acartia* sp. was $5.6 \pm 3.4\%$ immediately after exposure and $10.0 \pm 5.1\%$ 5 h after exposure (Fig. 5), while in the control treatment, only 1 individual died ($0.6 \pm 1.4\%$) (after 5 h). In *Calanus* sp., mortality was significantly higher in the pressure-exposed copepods compared to the control 5 h after treatment ($3.3 \pm 3.6\%$ vs. no mortality). Immediately after treatment, there was a tendency for higher mortality after pressure exposure, but no significant difference between treatments (pressure-exposed: $1.6 \pm 1.8\%$ vs. no mortality; Fig. 5; Table 2).

3.4. Behavior: swimming speed

The mean swimming speed was significantly lower after the pressure exposure treatment compared to the control treatment (Table 2). The mean swimming speed was higher in *Calanus* sp. than in *Acartia* sp. (Table 2; Fig. 6). In *Acartia* sp., the mean swimming speed of the pressure-exposed animals (0 h: $0.49 \pm 0.68 \text{ mm s}^{-1}$; 5 h: $0.52 \pm 0.71 \text{ mm s}^{-1}$ [mean \pm SD]) was significantly lower than in the control groups both immediately and 5 h after treatment (0 h: $1.12 \pm 0.77 \text{ mm s}^{-1}$; 5 h: $1.10 \pm 0.73 \text{ mm s}^{-1}$) (Fig. 6; Table 2). In *Calanus* sp., the swimming speed was lower in the pressure-exposed copepods ($2.64 \pm 1.28 \text{ mm s}^{-1}$) than

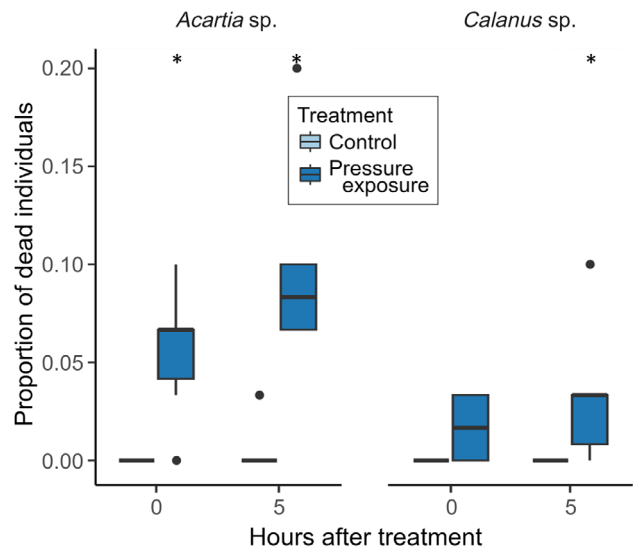


Fig. 5. Proportion of dead individuals of *Acartia* sp. and *Calanus* sp. after exposure to a control treatment or a pressure exposure treatment, measured 0 h and 5 h after treatment. The proportion is calculated from replicates ($n = 6$) with 30 individuals each. Significance is demonstrated with an asterisk (*) at the top of the graph. The horizontal middle line shows the median (25th and 75th percentile), and the whiskers display the minimum and maximum values within 1.5 times the interquartile range above/below the 75th/25th percentile. Potential outliers are shown as black dots

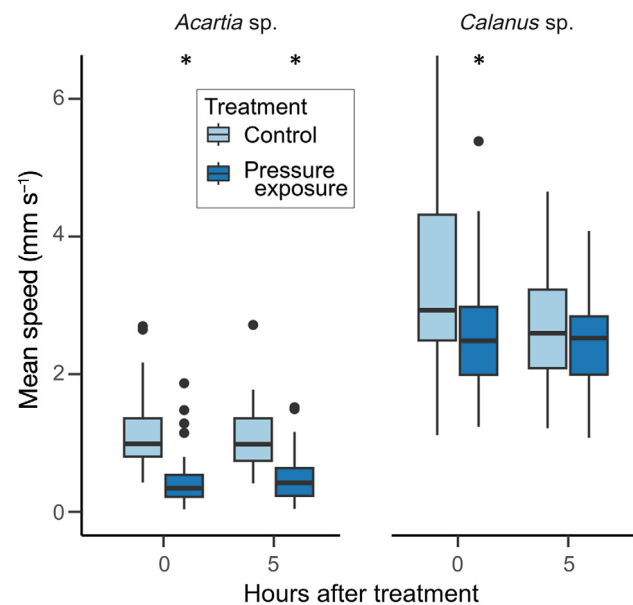


Fig. 6. Measured mean swimming speed (mm s^{-1}) of *Acartia* sp. and *Calanus* sp. after exposure to a control treatment or a pressure exposure treatment, measured 0 h and 5 h after treatment. The mean swimming speed was calculated from each dish (6 from each replicate, $n = 6$). Significance is demonstrated with an asterisk (*) at the top of the graph. Box plot description as in Fig. 5

in the control group ($3.32 \pm 1.68 \text{ mm s}^{-1}$) immediately after treatment. After 5 h, the mean swimming speed showed no differences (pressure exposure: $2.6 \pm 1.27 \text{ mm s}^{-1}$, control treatment: $2.71 \pm 1.16 \text{ mm s}^{-1}$). There was no significant difference in copepod prosome length between treatments for either species (*t*-test, *Acartia* sp., $p = 0.89$; *Calanus* sp., $p = 0.30$). During the 4 min of recording, the mean speed per minute remained stable for both species and time points after treatment (0 and 5 h).

4. DISCUSSION

This study demonstrates a method for re-creating a rapid pressure drop associated with seismic airguns in the laboratory. Although the size of the pressure drop was similar to the pressure drop of 20–60 m from a modeled airgun array, the speed of the drop was approximately 6 times slower (airgun array: 0.37 bar ms^{-1} ; Khodabandeloo 2018). Even so, the mortality rate of the pressure-exposed *Acartia* sp. was significantly affected by the re-created pressure drop. In contrast, the mortality rate of pressure-exposed *Calanus* sp. was only significantly affected after 5 h. Pressure-exposed *Acartia* sp. was also significantly less active than the control group up to 5 h after treatment, whereas behavior in *Calanus* sp. significantly differed between treatments only immediately after treatment. The potential expansion of microbubbles in animals was excluded as a potential cause of damage. Thus, a pressure drop alone can cause damage to zooplankton. The results of this study could help interpret those of previous studies that have investigated the effects of seismic exposure on mortality and behavior in zooplankton (McCauley et al. 2017, Fields et al. 2019, Vereide et al. 2023) by adding novel data on the characteristics of the sound field that could cause damage to zooplankton, and which taxa may be most vulnerable.

4.1. Effects on mortality and behavior in copepods

4.1.1. Mortality

Higher mortality was detected in pressure-exposed *Acartia* sp. compared to control groups both immediately and 5 h after treatment. In *Calanus* sp., there was a difference between pressure-exposed copepods and control only after 5 h, although a tendency for a similar effect was also observed immediately after treatment. The impact of seismic exposure on mortality

has previously been investigated in both *Calanus* sp. and *Acartia* sp. Fields et al. (2019) reported an immediate maximum mortality rate of 15% in airgun-exposed adult *C. finmarchicus*, which was distinctly higher than that of *Calanus* sp. in this study. The animals were exposed to a single shot from 2 small airguns (520 in^3 [-8521 cm^3] in total), but significant effects on mortality were detected only 5 m from the source (Fields et al. 2019). Investigating *A. tonsa*, Vereide et al. (2023) found an immediate mortality of ~14% in the naupliar stages of the copepod, also higher than that observed in our current study. Vereide et al. (2023) exposed the nauplii to the airguns for ~2.5 h, which was significantly longer than the exposure time in this study, where only a single pressure drop was applied. In contrast to both of these studies, McCauley et al. (2017) noted increased immediate mortality in natural zooplankton communities by up to 2.5-fold after exposure to a seismic transect compared to the control transects (~45 vs. ~20%). The re-created pressure drop of ~2 bar would occur in a sound wave with a peak pressure level of 226 dB re $1 \mu\text{Pa}$ (200 kPa) (ISO 2017). This is lower than the measured peak pressure of 1369 kPa (closest to the airguns) in Fields et al. (2019) but higher than that reported by Vereide et al. (2023) (48.9 kPa, 50 m from the source) and McCauley et al. (2017) (1.4 kPa, 509–658 m). Our results indicate that only lower levels of mortality may be expected even for the more sensitive *Acartia* sp. However, the duration of the exposure was short, and longer exposure may lead to increased mortality (Kok et al. 2023).

The mortality rate in pressure-exposed *Calanus* sp. increased from 0 h to 5 h, whereas it was already elevated in *Acartia* sp. immediately after treatment. This observation suggests that mortality may become apparent after a longer duration, similar to the delayed mortality observed in *Acartia* nauplii following seismic exposure (Vereide et al. 2023). Hence, it is important to exercise caution and avoid underestimating the potential mortality over time, despite the initially low mortality rates reported in this and other studies (Pearson et al. 1994, Parry et al. 2002, Fields et al. 2019).

4.1.2. Swimming behavior

The swimming activity in pressure-exposed *Acartia* sp. was lower both 0 h and 5 h after treatment, whereas decreased activity was only detected in *Calanus* sp. immediately after treatment. The effects of seismic exposure on zooplankton behavior are widely unknown, both individually and at a popula-

tion level, and although some studies have looked into behavioral impact in zooplanktonic species from anthropogenic underwater noise (Aspirault et al. 2023), few studies have examined behavioral effects in copepods (Vereide & Kühn 2023). For example, Fields et al. (2019) found no effects on the escape responses in *C. finmarchicus*. They measured escape performance and changes in the sensory threshold needed to initiate an escape but reported no difference for any distance to the airgun. To our knowledge, no studies have examined the effects of seismic exposure on the behavior of *Acartia* sp. However, Kühn et al. (2023) reported that the feeding rate of *A. tonsa* decreased when exposed to boat noise, a continuous sound source. Thus, different species might react differently to exposure of seismic airguns or anthropogenic sound. The few studies conducted also highlight the need for more knowledge on behavioral effects after noise exposure.

On a larger scale, McCauley et al. (2017) observed a 'hole' in the zooplankton layer after exposure to a seismic airgun. Using a 120 kHz sonar frequency, they were unable to observe individual zooplankton, but they could detect larger aggregations. They suggested that the 'hole' is due to changes in zooplankton orientation or a spread in the zooplankton mass. Furthermore, zooplankton may be disabled in their sensory capacity, resulting in behavioral changes and sinking of the animals (McCauley et al. 2017). Although our study did not measure sinking, it demonstrated overall less swimming activity in *Acartia* sp. than in *Calanus* sp. Therefore, the differences observed in the results of previous studies might indicate that smaller copepods or species similar to *Acartia* sp. may exhibit less movement after seismic exposure compared to larger copepods like *Calanus* sp. Furthermore, the absence of significant differences in activity between the pressure-exposed and control group after 5 h, as opposed to immediately after exposure, in *Calanus* sp., could indicate a recovery process. This suggests that the animals may experience effects only in the short term following exposure but manage to recover within a few hours.

It is noteworthy that the swimming activity was measured in a 2-dimensional setup, and it may not directly reflect real-life scenarios. However, the primary focus of this study was to compare the differences between exposure and control groups, as well as variations between different species. Consequently, the study highlights the significance of investigating potential airgun characteristics and species-specific responses, rather than providing data on natural swimming speeds.

4.2. Differences between species

Both species used in this study are commonly used in laboratory and field experiments (Jonsson & Tiselius 1990, Hygum et al. 2000, Aarflot et al. 2018, Rotolo et al. 2021). The species differ in their physiological and functional traits despite being visually similar. First, *Calanus* sp. is a more lipid-rich species, containing proportionally more lipids per unit body area than *Acartia* sp. (Lee et al. 2006). Studies have suggested that changes in pressure may harm animals that have areas of different densities in their bodies, e.g. lipid storage vacuoles (Bamber & Seaby 2004). Zarubin et al. (2016) also detected a pattern of greater effects of pressure change on the grazing rate of lipid-rich copepods. However, this contrasts with the results of this study, in which *Calanus* sp. experienced fewer effects on mortality and fewer changes in swimming activity after a pressure drop than *Acartia* sp. Second, the species differ in their behavioral patterns, such as swimming and escape strategies. For example, *Calanus* sp. has a higher velocity and beat cycle (leg stroke duration and pause) than *Acartia* sp. (Kjørboe et al. 2010b). While this distinction may not directly influence long-term mortality or behavioral alterations, it could potentially help explain why the behaviors of different species are affected in distinct ways. These findings suggest that *Acartia* sp. experience a greater impact on the swimming activity compared to *Calanus* sp., raising the possibility that *Acartia* sp. may be more sensitive to exposure or that the effects on swimming activity are more pronounced following exposure.

Furthermore, copepods possess external sensory hair and mechanoreceptive setae (Gassie et al. 1993), and respond to hydrodynamic stimuli, with which behavioral responses can be altered (Lenz & Hartline 2015). Behavioral responses, the ability to modify behavioral patterns, and sensitivity to external signals can vary among species (Kjørboe et al. 2018). The observed differences could indicate that *Calanus* sp. have a wider sensory system and can potentially use other mechanisms if exposed to pressure changes. Furthermore, low-frequency sound can cause damage in the sensory setae of copepods (Solé et al. 2021), which can differ between species (Fields 2014). Lastly, as previously mentioned, McCauley et al. (2017) detected a substantial increase in mortality after airgun exposure. Interestingly, they reported that the group of dead copepods was dominated by smaller species (*A. tranteri*, *Oithona* spp.), similar in size to *Acartia* sp. used in this study. Overall, the variations observed in the effects on swimming activity

suggest that different species may be influenced in distinct ways, potentially due to their varying morphologies or behavioral patterns. To determine which morphological characteristics are most susceptible and the underlying reasons, further specialized investigations are required.

4.3. Pressure drop as a potential cause of damage

The study demonstrated that the pressure drop around a seismic airgun array can trigger cavitation only in a small area around the source. The majority of the water surrounding the source experiences pressure increase and decrease without cavitation, even in the region where a 2 bar pressure drop is observed. Thus, this experiment reflects the region between 20 and 55 m around a typical airgun array. The pressure drop we re-created was between 3 and 1 bar absolute pressure. It has previously been speculated that the high positive peak followed by a high negative peak (peak pressure gradient), similar to the pressure drop in this study, may cause physical damage to some marine taxa (McCauley & Duncan 2017, McCauley et al. 2021). Ideally, negative pressure should therefore have been used; however, achieving this in a laboratory setting is very challenging. Consequently, the pressure was slowly increased and then rapidly released to create a pressure drop.

Liquids, known for their high incompressibility, maintain a relatively constant volume under compression. The bulk modulus of a liquid, which measures its compressibility, can be defined as:

$$K = -dp/(dV/V_0) \quad (2)$$

where K (N m^{-2}) represents the bulk modulus, dp is the applied pressure changes on the liquid, dV is the change in volume, and V_0 denotes its initial volume (Munson et al. 2002). The bulk modulus is a characteristic of a liquid and varies with temperature. At 15°C , the bulk modulus of seawater is approximately 2.34 GPa, while that of paraffin oil is around 1.66 GPa. Paraffin oil can serve as an analog for lipids found in copepods. It is worth mentioning that although these liquids exhibit substantial resistance to pressure, their volume experiences slight fluctuations under high-pressure conditions. Therefore, when a liquid organism with an elastic shell experiences an increase in hydrostatic pressure, the elastic shell undergoes compression to counteract the volume reduction of the organism. On the other hand, liquids typically have negligible tensile strength. That is, when the pressure drops and the

volume of the organism returns to its initial value, there is minimal resistance from the liquid inside the organism, and only the elastic shell experiences tensile stress. This way, a pressure drop could harm the elastic shell, which could potentially lead to physical damage, or even mortality. It is important to note that the pressure drop occurred independently of any generation of acoustic waves within the pressure tube, resulting in the absence of particle velocity that would normally be associated with the acoustic waves. This absence of particle motion is a considerable advantage in the experimental setup. It facilitates the isolation of the effects of the rapid pressure drop on zooplankton. Many studies on the effects of underwater sound on invertebrates emphasize particle motion (Nedelec et al. 2016). In this study, we show that a pressure drop itself can impact zooplankton. Future studies could focus on gaining a better understanding of the mechanisms behind this damage.

Similar methods of exposing zooplankton to pressure changes have previously been adopted for hydrostatic pressure changes over a longer time and with lower pressure (e.g. in relation to diel vertical migration) (Zarubin et al. 2016). Investigating effects of real seismic surveys are often expensive and logistically difficult. Furthermore, it is close to impossible to isolate certain characteristics underlying potential damage when conducting field experiments. Therefore, the pressure tube offers a unique method of testing how zooplankton may be damaged from seismic surveys.

4.4. Transferrable to real-life settings?

This study isolated and re-created a rapid pressure drop similar to those observed close to airguns. From the measured and modeled pressure variations, we can estimate at which range from a real-life airgun array such pressure drops occur.

The comparison between the re-created pressure drop and that of an airgun array (2730 in^3) demonstrates that the hydrostatic pressure drop of ~ 2 bar would not occur at distances further away than ~ 55 m from the airgun (vertically, below the airgun). This suggests that the mortality found in this study corresponds to the studies reporting effects in relatively close distance from an airgun array. However, because the pressure drop in the laboratory was slower than the pressure drop that occurs in the sound field around a real airgun array, the range at which damage occurs may be larger in the field.

Although we modeled a typical airgun array, seismic surveys may operate with arrays of total chamber volume up to 5300 in³ (~86 851 cm³) (Hovem & Tronstad 2012, Slabbekoorn et al. 2019). In that case, the pressure drop of ~2 bar may in other cases extend beyond the distances modeled in this study. However, even a tenfold increase in range compared to the range modeled here would only amount to a 600 m range around the airgun array. Thus, our results corroborate findings from studies that find low levels of mortality close to airgun arrays (Pearson et al. 1994, Fields et al. 2019, Vereide et al. 2023), but they cannot explain mortality at larger distances of up to 1 km from the source as found in McCauley et al. (2017).

4.5. Conclusions

This study demonstrated a novel setup to expose zooplankton to a rapid pressure drop associated with seismic airgun arrays at close range, and showed that such a rapid pressure drop can negatively affect zooplankton mortality and behavior. Moreover, our results indicate that the pressure drop itself can cause damage, independently from cavitation and particle motion. The results also show that *Acartia* sp. is more sensitive to this pressure drop than *Calanus* sp. This targeted focus solely on the pressure drop enables us to precisely identify its effects on zooplankton, eliminating potential confounding variables associated with particle motion. All in all, this study shows how a single characteristic of the sound field around an airgun array may cause damage to zooplankton, at what distance this is likely to happen, and that such effects can be species-specific. These data can be used to design future studies on the effects of anthropogenic noise on zooplankton, and shed light on the mechanisms behind how damage can occur.

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Appendix.

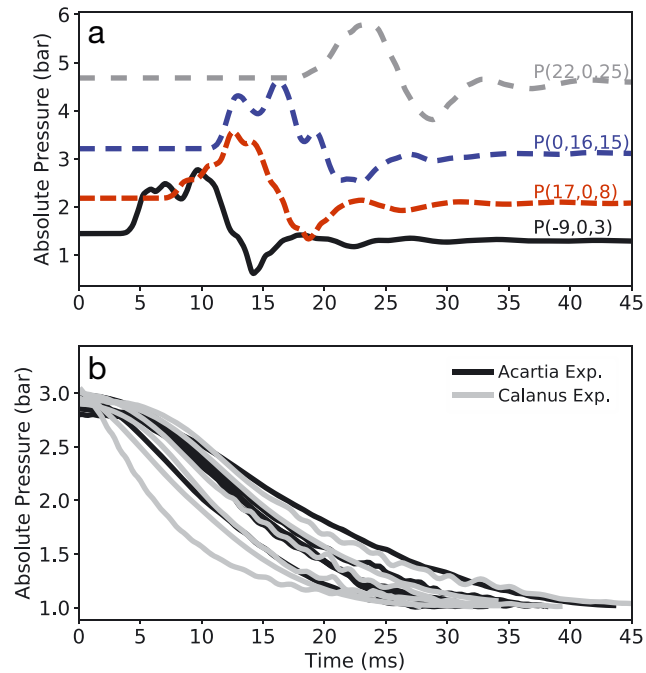


Fig. A1. (a) Modeled absolute pressure variation (bar) at 4 different points located at (x, y, z) around the 2730 in^3 ($\sim 44737 \text{ cm}^3$) airgun array (x [m], the direction the airgun travels; y [m], starboard direction; and z [m], the water depth measured from the water surface). (b) Maximum absolute pressure following the rapid release in the pressure exposure treatments in *Acartia* sp. and *Calanus* sp.

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