



# Effects of airgun discharges used in seismic surveys on development and mortality in nauplii of the copepod *Acartia tonsa*<sup>☆</sup>

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## ABSTRACT

Seismic surveys are conducted worldwide to explore for oil and gas deposits and to map subsea formations. The airguns used in these surveys emit low-frequency sound waves. Studies on zooplankton responses to airguns report a range of effects, from none to substantial mortality. A field experiment was conducted to assess mortality and naupliar body length of the calanoid copepod *Acartia tonsa* when exposed to the discharge of two 40-inch airguns. Nauplii were placed in plastic bags and attached to a line at a depth of 6 m. For each treatment, three bags of nauplii were exposed to one of three treatments for 2.5 h: Airgun array discharge, a boat control, or a silent control. After exposure, nauplii were kept in filtered seawater in the laboratory without food. Immediate mortality in the nauplii was approximately 14% compared to less than 4% in the silent and boat control. Similarly, there was higher mortality in the airgun exposed nauplii up to six days after exposure compared to the control treatments. Nearly all of the airgun exposed nauplii were dead after four days, while >50% of the nauplii in the control treatments were alive at six days post-exposure. There was an interaction between treatment and time on naupliar body length, indicating lower growth in the nauplii exposed to the airgun discharge (growth rates after 4 days: 1.7, 5.4, and 6.1  $\mu\text{m d}^{-1}$  in the airgun exposed, silent control, and boat control, respectively). These experiments indicate that the output of two small airguns affected mortality and growth of the naupliar stages of *Acartia tonsa* in close vicinity to the array.

## 1. Introduction

Anthropogenic underwater noise, such as that generated by boat traffic or seismic surveys, is an increasingly important source of environmental stress for marine organisms (Hildebrand, 2009; Fritschi et al., 2011; Swaddle et al., 2015; Cox et al., 2018; Duarte et al., 2021). In the Norwegian Exclusive Economic Zone alone, more than 18000 vessel  $\text{km}^2 \text{yr}^{-1}$  are covered by seismic surveys (NDP, 2021). Seismic airguns rapidly release high-pressure air (airgun discharge) to generate an acoustic signal dominated by frequencies lower than 200 Hz, and their output has been recorded over 4000 km away from the source (Kavanagh et al., 2019).

Most studies on the possible impacts of seismic surveys on marine organisms have focused on fish and mammals (Gordon et al., 2003; Slabbekoorn et al., 2019). Seismic surveys interfere with the social interactions, behavior, and orientation of marine mammals and fish with well-developed auditory systems (Engås et al., 1996; Gordon et al., 2003; Cox et al., 2018; Popper and Hawkins, 2019). Some studies have also demonstrated increased mortality and damage in fish (Carroll et al., 2017) and fish larvae (Kostyuchenko, 1973; Booman et al., 1996) exposed to airgun discharges.

Few studies have investigated the effects of seismic survey activity on zooplankton (Solé et al., 2023). The results are contradictory and suggest species- and stage-specific responses and the need for standardized

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**Table 1**  
Summary of previous studies on the effects of seismic surveys on zooplankton.

Study	Zooplankton group	Measured parameters	Level of effects	Sound type	Received sound level (MD/MS) <sup>a</sup>		Distance from the source (m)	Size and number of airgun (s) (vol/pressure)
					Sound pressure (kPa) (ptp/ztp) <sup>b</sup>	SEL (dB re 1 $\mu\text{Pa}^2$ s)		
<b>Copepods:</b>								
McCauley et al., 2017	Various, <i>in situ</i> samples	Mortality	High	Live	1.4 (509–658 m), 0.8 (1.1–1.2 km) (MS) (ptp)	156 (509–658 m), 153 (1.1–1.2 km) (MS)	71–1300	1*150 inch <sup>3</sup> /13.8 MPa
Fields et al., 2019	Large copepods ( <i>Calanus finmarchicus</i> )	Mortality, behavior, gene expression	Little to no effect	Live	1363 (0 m) (MS) (ztp)	221 (0 m), 183 (25 m) (MS)	0–25	2*260 inch <sup>3</sup> /13–14.4 MPa
This study	Small copepods ( <i>Acartia tonsa</i> )	Mortality, growth	Little	Live	48.9 (50 m) (MS) (ptp)	180 (50 m), 166 (1100 m) (MS)	50–1200	2*40 inch <sup>3</sup> /11 MPa
<b>Bivalve larvae:</b>								
Parry et al., 2002	Scallop larvae (and adult)	Mortality, population/catch rates	No effect	Live	Not given	Not given	~11–600	Airgun (24) array of total 3542 inch <sup>3</sup> /not given
de Soto et al., 2013	Scallop larvae	Development, abnormalities	High	Recorded	Not given	161 to 165 (MD)	0.05–0.1	Airgun array of total 6920 inch <sup>3</sup> /not given
<b>Other crustacean larvae:</b>								
Pearson et al., 1994	Crab larvae (zoea)	Mortality and development	No effect	Live	316.2 (max, 1 m) (MS) (ptp)	Not given	1–10	7 airguns, total volume 840 inch <sup>3</sup> /not given
Day et al., 2016 <sup>c</sup>	Lobster larvae	Fecundity, morphology, competency	No effect	Live	141.3 to 223.9 (MS) (ptp)	186 to 190 (max) (MS)	~0–1000	1*45–150 inch <sup>3</sup> /13.8 MPa (max)

<sup>a</sup> MD: Modeled, or MS: Measured.

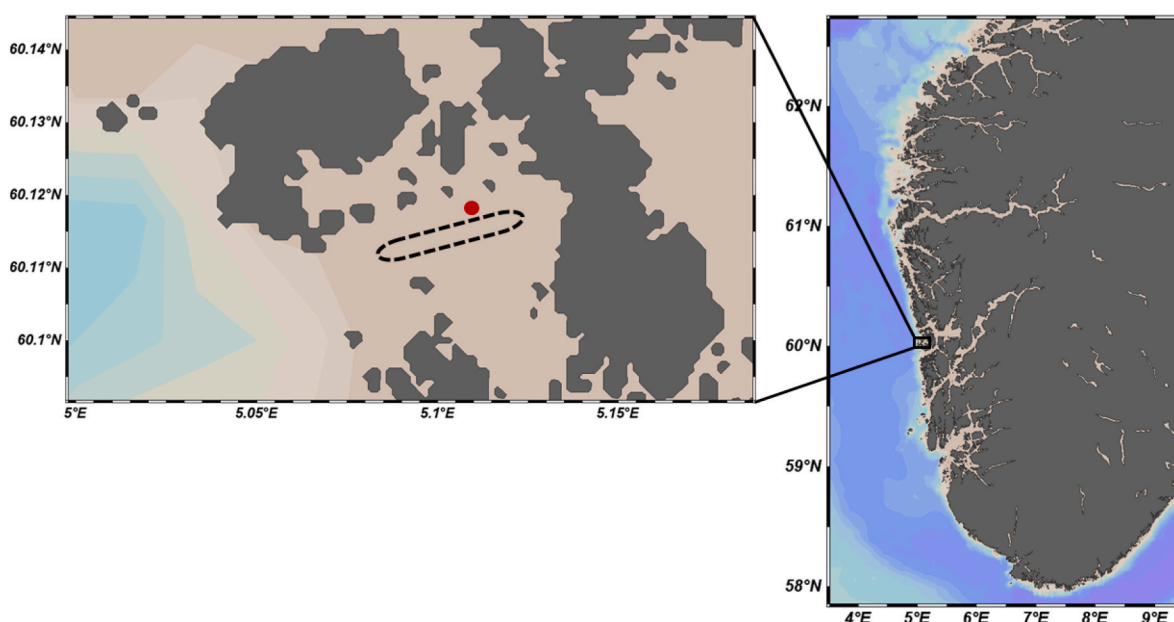
<sup>b</sup> The sound pressure levels (kPa) were given as either peak-to-peak (ptp), or zero-to-peak (ztp). From the studies demonstrating sound pressure levels in dB, the calculation to Pa was made using the following equation.

$$p(\text{Pa}) = p_0 \cdot 10^{\frac{L_p(\text{dBSPL})}{20}}$$

<sup>c</sup> The adult females were exposed, whereas the effects were measured in hatched larvae.

methodological approaches (Table 1). Fields et al. (2019) reported a small, localized impact from the discharge of a two airgun array on the copepod *Calanus finmarchicus*, a key species in North Atlantic waters (Fields et al., 2019). The immediate mortality increased by less than 10% in animals close to the airguns (<5 m), followed by a nominal

increase in mortality 7 days after exposure, but again, only at close range (<5 m). The zoea of Dungeness crab (*Metacarcinus magister*) experienced only 0–2% immediate mortality and no longer-term effects of exposure to airgun discharges (Pearson et al., 1994). In contrast, McCauley et al. (2017) reported a two- to three-fold increase in dead zooplankton, both



**Fig. 1.** Study area. On the left, the location where the zooplankton were exposed is indicated with a red circle. A dotted black line denotes the transect of the vessel towing the airgun array. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**

Environmental conditions during the field experiment to expose *Acartia tonsa* nauplii to a silent control, a boat control, or an airgun array discharge. The temperature and light at 6 m depth are the averages during the exposure time. \*The bags in the airgun array treatment were deployed in the water at 16:00, but the airgun array shooting did not start until 17:00.

Date	Treatment	Nauplii/bag (mean $\pm$ SD)	Bags exposure time	Secchi depth (m)	Temp. surface ( $^{\circ}$ C)	Temp. 6 m ( $^{\circ}$ C)	Light 6 m (lux, lm/m <sup>2</sup> )
15.2.21	Airgun array	1500 $\pm$ 156	17:00*-19:35	11.0	2.5	3.1	76
16.2.21	Boat control	2014 $\pm$ 253	14:01–16:45	13.0	2.4	3.3	379
19.2.21	Silent control	331 $\pm$ 130	10:16–13:00	11.0	2.4	3.2	1066

larvae and adults, after exposure to one airgun in the field. These negative impacts were observed up to the maximum sampling range of 1200 m (McCauley et al., 2017).

In addition to the direct effects of the airgun discharge on mortality, sublethal effects include altered developmental rates and morphological deformities. For example, snow crab (*Chionoecetes opilio*) eggs (Christian et al., 2003), lobster juveniles (*Jasus edwardsii*) (Day et al., 2022), and larval scallops (*Pecten novaezelandiae*) (de Soto et al., 2013) developed slower than the control groups after exposure to seismic airguns and exhibited morphological abnormalities. These effects are species- and stage-specific, as is also the case for mortality (Pearson et al., 1994; Day et al., 2016).

Given the limited number of studies on the effects of airgun exposure on zooplankton, particularly on the juvenile stages, and the contradictory results of the few published reports, more knowledge is needed to understand potential impacts. This study aimed to test the effects of airgun discharge on immediate and delayed mortality and growth in the nauplii of a marine copepod (*Acartia tonsa* Dana).

## 2. Materials and methods

The effects of airgun array discharges used in seismic surveys were investigated by exposing *Acartia tonsa* nauplii to the discharge of an airgun array in a controlled experiment with three treatments (silent control, boat control, and airgun array). After exposure, immediate and delayed mortality (1–6 days after exposure) and naupliar growth were investigated. The experiments were conducted during winter 2021 at Austevoll Research Station (Institute of Marine Research, Norway), and copepods were exposed to the discharge of a seismic airgun array in Bakkasund, Austevoll (60.116667 $^{\circ}$ N, 5.11 $^{\circ}$ E) (Fig. 1, Table 2).

### 2.1. Experimental animals

*Acartia tonsa* is an epipelagic calanoid copepod that is commonly

found in Norwegian waters and is widely distributed worldwide, especially in near-shore and estuarine areas (Cervetto et al., 1999). This species has a high tolerance to salinity and temperature changes (Sunar and Kir, 2021). In the North Atlantic coastal waters, *Acartia* sp. is frequently among the common zooplankton in spring and summer and serves as an important food source for many commercial fish species (Sullivan et al., 2007).

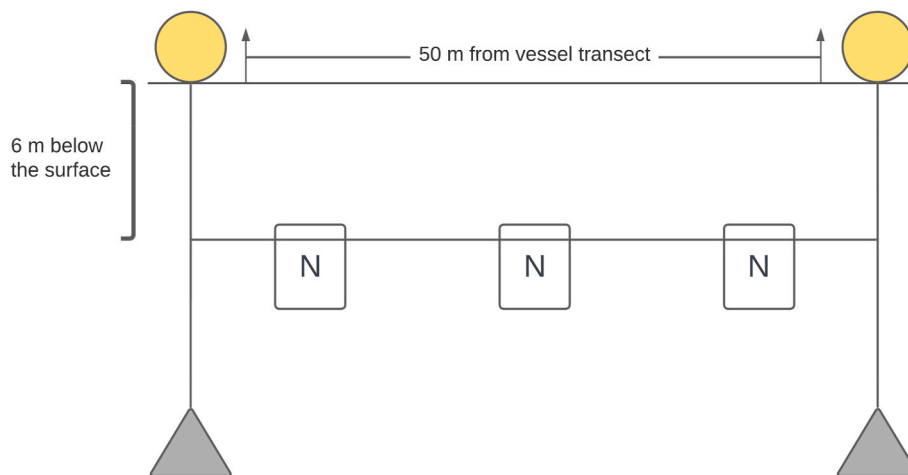
### 2.2. Egg hatching

Several cultures of *Acartia tonsa* were established from resting eggs (sourced from C-Feed AS; Trondheim, Norway). To produce nauplii for the experiments, a portion of the eggs was incubated in 80 L tanks with 0.2  $\mu$ m filtered seawater (FSW) at 21  $^{\circ}$ C under heavy aeration until hatching (according to the manufacturer's instructions). After 24 h, nauplii were transferred to gently aerated 5 L tanks, in which the temperature was slowly adjusted ( $-0.3$   $^{\circ}$ C h<sup>-1</sup>) to 6  $^{\circ}$ C (i.e., the temperature in the sea at the exposure site) over the next 48 h (Table 2).

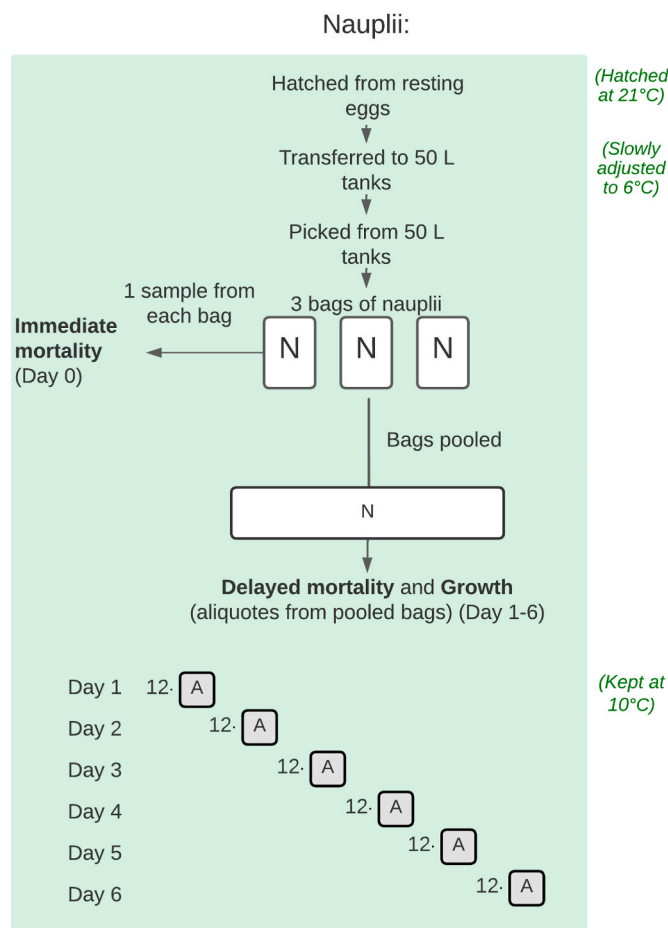
### 2.3. Experimental setup

#### 2.3.1. Before exposure

On each treatment day (Table 2), newly hatched nauplii were transferred from the culture to three experimental bags (50  $\times$  18 cm). Because of the short hatching time it was necessary to use different batches of eggs each day (i.e., for each treatment). These batches varied in the number of nauplii produced. Therefore, the number of nauplii differed between treatments (Table 2). Bags (~1 L) were filled with 0.2  $\mu$ m FSW with no head space. The bags were sealed, attached at 0.5 m intervals to an 8-m long line, and transported in a soundproof box to the experimental site (Figs. 1 and 2). A temperature/light data logger (HOBO Pendant<sup>®</sup> MX) was attached to this line. The line was suspended at a depth of 6 m between two buoys at a distance of 50 m from one edge of the transect of the vessel towing the seismic airgun array (Figs. 1 and



**Fig. 2.** A schematic diagram showing the field setup used to expose *Acartia tonsa* nauplii to either a silent control, a boat control, or an airgun array discharge. Buoys (circles) with attached weights (triangles) were located at a 50 m distance at the closest, parallel to the ship transect. The line with the bags was attached at 6 m depth. The bags were attached to the line every 0.5 m.



**Fig. 3.** Overview of the number of bags, samples, and endpoints of nauplii of *Acartia tonsa* for each treatment (silent control, boat control, airgun array). Immediate mortality was measured from 3 replicates (one from each bag) immediately after exposure. Delayed mortality and growth were measured from 12 aliquots from the pooled bags for each treatment and each day after exposure (1–6 days).

**Table 3**

Overview of statistical tests. a) Binomial GLM on the effect of treatment (silent control, boat control, airgun array) on immediate mortality in *Acartia tonsa* nauplii ( $n = 9$ ), and b) Kruskal-Wallis on the effect of treatment on the proportion of dead nauplii for each day separately, up to 6 days after exposure (delayed mortality) ( $n_{\text{aliquots per day}} = 29\text{--}36$ ). c) LMM on the effect of treatment (silent control, boat control, airgun array) on individual *Acartia tonsa* body length ( $\mu\text{m}$ ) ( $n_{\text{individual}} = 356$  (silent control), 392 (boat control), 167 (airgun array)), and d) Pearson's Correlation Coefficient test on the correlation between body length and developmental stages in nauplii ( $n_{\text{individual}} = 915$ ).

	Effects	Estimate	SE	z	P
a) Immediate mortality	(Intercept)	3.34	0.18	18.82	<2e-16
	Treatment: Boat	0.22	0.20	1.11	0.27
	Treatment: Airgun array	-1.60	0.19	-8.53	<2e-16
b) Delayed mortality	<b>Days after treatment</b>	-	-	$\chi^2$	<b>P</b>
	Day 1	-	-	27.87	8.90e-07
	Day 2	-	-	16.43	2.70e-04
	Day 3	-	-	22.44	1.34e-05
	Day 4	-	-	23.04	9.95e-06
	Day 5	-	-	32.56	8.51e-08
	Day 6	-	-	22.53	1.28e-05
c) Body length	<b>Effects</b>	<b>Estimate</b>	<b>SE</b>	<b>95% CI (min/max)</b>	
	(Intercept)	124.028	2.47	119.27/128.80	
	Treatment: Boat	-5.57	3.43	-12.20/1.05	
	Treatment: Airgun array	-4.61	3.88	-12.13/2.89	
	Day	5.40	0.90	3.66/7.14	
	Interaction of Boat with Day	0.70	1.27	-1.75/3.16	
Interaction of Airgun array with Day	-3.69	1.47	-6.53/-0.84		
d) Body length and stage		<b>Correlation coefficient</b>		<b>Df</b>	<b>P</b>
		0.91		903	<2.2e-16

2).

**2.3.2. Treatments**

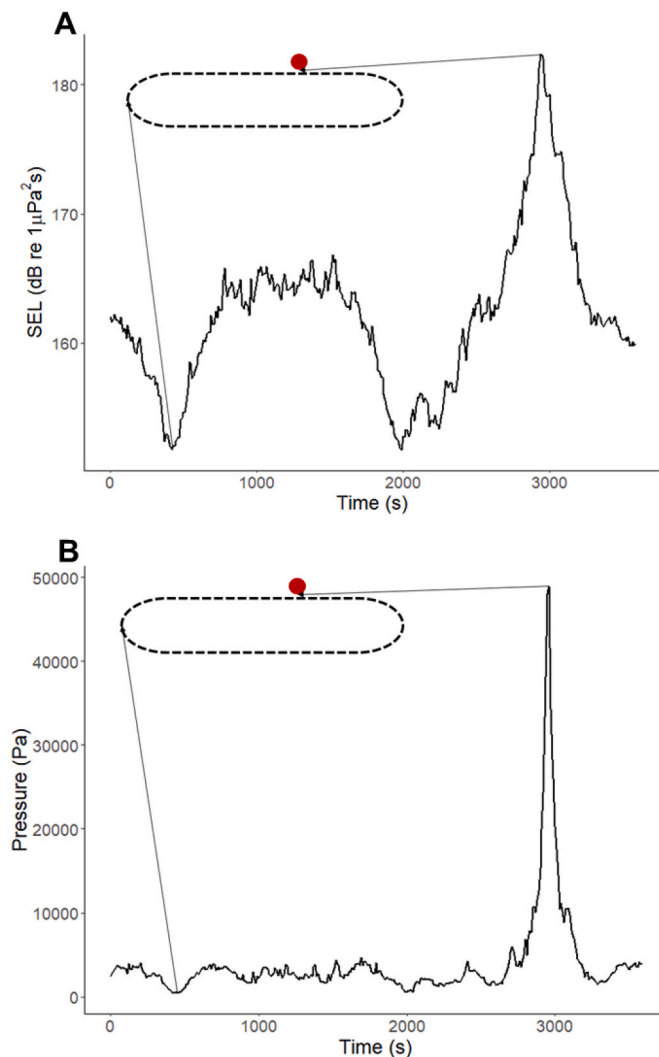
The experiment consisted of three treatments: Exposure to multiple discharges from two airguns (the “airgun array”), a boat control, and a silent control. The airgun array treatment consisted of two small airguns (40 inch<sup>3</sup>, HGS Sleeve Guns) fired at a pressure of 110 bar every 10 s for 3 h. The airgun array was towed at 2 knots at a depth of 3–4 m along an oval transect (3 nmi in total) by the research vessel “H. U. Sverdrup II”, for another study (McQueen et al., 2022). The vessel sailed between 50 m and 1220 m from the line holding the bags (Fig. 1). During the boat control treatment, H.U. Sverdrup followed the same transect without discharging the airguns. The boat control treatment was included to control for the sound generated by the vessel itself. During the silent control treatment, H.U. Sverdrup was outside the area. Exposure lasted for approximately 2.5 h (see Table 2 for details). After each exposure (Table 2), bags for each treatment were recovered and transported to the laboratory in a sound-absorbing box. During all treatments, the weather was partly cloudy, wind speed was 6–7 m/s, and wave height was 0.5–1 m. The air temperature ranged from 7 to 12 °C during all three days.

**2.4. Analysis of animals exposed to the different treatments**

Copepods were counted and sorted using a Leica stereomicroscope (Leica Microsystems, Switzerland, Stereozoom S9i). Morphometrics were measured from photos taken with an AirLab 2.0 Leica Microsystems equipped with a Leica CLS150 LED light.

**2.4.1. Naupliar mortality**

Immediate mortality of the nauplii was measured within 2 h of returning to the laboratory (<4 h after exposure). Samples (50–100 ml) from each nauplii bag were removed and the number of live and dead individuals was quantified under a stereomicroscope (Olympus SZX10), carefully stimulating the animals with water movements to check for signs of life. To investigate delayed mortality for each treatment, 72 aliquots of 8 nauplii each ( $7.5 \pm 2.2$ ) were transferred to individual 15 ml-wells (6 wells/plate, Nunc A/S Denmark) with 0.2  $\mu\text{m}$  FSW. The well plates were incubated at 10 °C to limit the increase in temperature after exposure under a 12:12 h light-dark cycle for 1–6 d after exposure. No food was added during this experiment to avoid confounding effects of



**Fig. 4.** Sound pressure levels during the airgun array exposure. A) The sound exposure level (SEL) and B) the peak-to-peak sound pressure level (Pa) as a function of time (s) during the airgun array exposure. The dotted line indicates the trajectory of the vessel, and the red dot indicates the location of the nauplii, from where the arrows demonstrate the sound pressure level and sound exposure level at selected distances from the vessel. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

variation in feeding between individuals and/or aliquots. Within the experimental time of six days, some individuals would most likely develop into stage III (at which *Acartia tonsa* nauplii begin to feed) and, therefore, these nauplii would eventually die of starvation. However, since the study's objective was to test the effect of treatment on the first naupliar stages, and not on later development, this did not impact the evaluation. Dead and live nauplii were counted on each consecutive day in 12 of the 72 wells: 12 new wells each day (Fig. 3). After counting, the developmental stage of the nauplii was scored, the nauplii were photographed, and body length was measured from the photos.

#### 2.4.2. Body length and development

The naupliar developmental stage of both dead and live individuals was scored according to Murphy and Cohen (1978), and body length ( $\mu\text{m}$ ) was measured from the micrographs using ImageJ (ImageJ 1.53e) (Schneider et al., 2012). Body length and developmental stage were measured for all individuals from days 1–6 after exposure. However, as all airgun-exposed individuals had died by day 5 and only live animals

were included in this analysis, differences in growth and development between the treatments could only be tested for days 1 to day 4. Growth rates were calculated from model estimates (described below). Because different individuals were measured at each time point, growth rates represent the average body weight/length differences over time.

#### 2.5. Data analysis

All data analyses were conducted using the R statistical software (R Core Team, 2022). Before conducting statistical tests, all data were graphically assessed for normality. The effect of treatment on immediate mortality was tested using the proportion of live nauplii versus the proportion of dead nauplii (Fig. 3) as a dependent variable and treatment (silent control, boat control, airgun array) as a fixed effect in a binomial GLM ( $n = 9$ , one subsample obtained from each of the three bags for each treatment) (Fig. 5) (Table 3). It was not possible to test the effect of treatment on delayed mortality using a binomial GLM because of overdispersion. Therefore, we tested the effect of treatment on the proportion of dead individuals using a Kruskal-Wallis test for each day separately (1–6 days after exposure; 29–36; aliquots per day) (Fig. 5) (Table 3). The number of nauplii within each aliquot (individual wells) was  $7.5 (\pm 2.2)$ . Aliquots with  $<5$  individuals (9.5% of the samples) were excluded from the analyses, therefore, the total number of aliquots per treatment varied ( $n_{\text{aliquot}} = 64$  (silent control), 66 (boat control), 61 (airgun array)). The effect of treatment on naupliar body length ( $\mu\text{m}$ ) was tested using a Linear Mixed-Effects Model (LMM). In this model the body length for each individual is a dependent variable, with aliquot as a random factor, and the treatment (silent control, boat control, airgun array), the number of days after exposure (days 1–4), and the interaction between these two variables, as fixed effects (Table 3) ( $n_{\text{individual}} = 356$  (silent control), 392 (boat control), 167 (airgun array);  $n_{\text{aliquot}} = 48$  (silent control), 48 (boat control), 45 (airgun array)) (Fig. 6, Table 3). The relationship between body length and developmental stage (NI–NIV) was tested using Pearson's Correlation Coefficient Test on all individuals. ( $n_{\text{individual}} = 915$ ). For all analyses, the model assumptions were verified by a visual assessment of the fitted values versus the residuals.

### 3. Results

#### 3.1. Sound measurements

The sound pressure level (kPa) ranged from 0.420 kPa at the furthest distance from the nauplii ( $\sim 1200$  m) to a maximum of 48.90 kPa at the closest distance (50 m) (Fig. 4A). The sound exposure level (SEL) was 152 dB re  $1 \mu\text{Pa}^2 \text{s}$  furthest away and 183 dB re  $1 \mu\text{Pa}^2 \text{s}$  at the closest distance (Fig. 4B). The sound exposure levels were reported for each second, the seconds without airgun blasts corresponded to the sound level in the boat control. Ambient sound levels during the experiment were between approximately 40 and 100 dB re  $1 \mu\text{Pa}^2 \text{s/Hz}$  (McQueen et al., 2022).

#### 3.2. Mortality

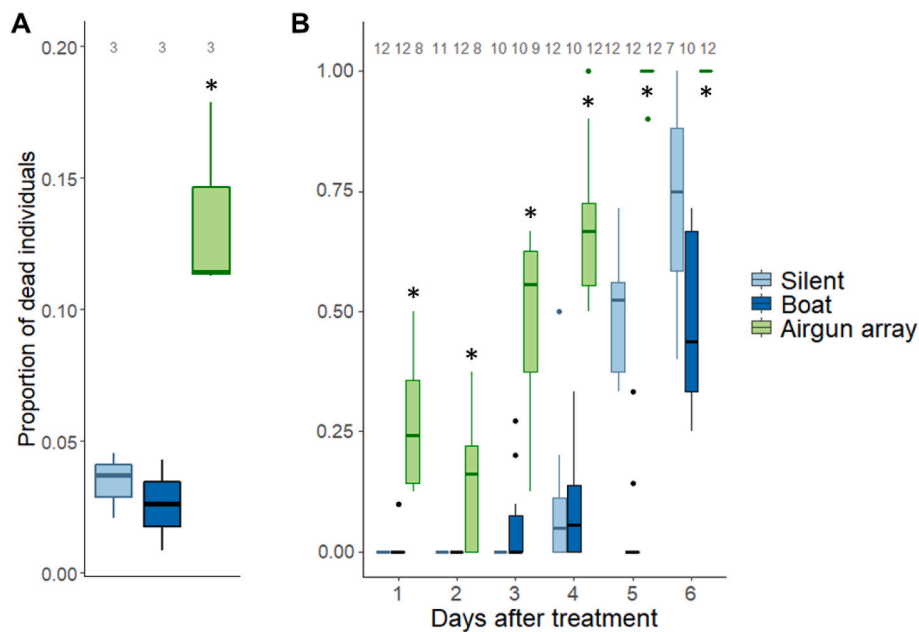
##### 3.2.1. Immediate mortality of nauplii

The immediate mortality was significantly higher in the airgun array treatment than in the silent and boat control treatments (Fig. 5A, Table 3). On average,  $13.5 \pm 3.8\%$  of the nauplii in the airgun array exposed treatment died immediately after exposure, compared to  $3.4 \pm 1.3\%$  in the silent control and  $2.6 \pm 1.7\%$  (mean  $\pm$  SD) in the boat control.

##### 3.2.2. Delayed mortality of nauplii

There was a significant difference in delayed mortality between the treatments (Fig. 5B, Table 3). On days 1 and 2 after exposure, nearly all of the nauplii from the control groups were alive, while  $27.0 \pm 14.2\%$





**Fig. 5.** The mortality of *Acartia tonsa* nauplii after experimental exposure to a silent control, a boat control, or an airgun array. A) Immediate mortality within 4 h of exposure, and B) delayed mortality from days 1–6 after exposure. Significance between treatments is noted with black asterisks. The total number of aliquots counted for each treatment per day is noted at the top of the figure. The horizontal lines indicate the median, and boxes indicate the 25th percentile (lower quartile), and the 75th percentile (upper quartile).

and  $14.8 \pm 14.2\%$  of the airgun array exposed nauplii had died in the aliquots from day 1 and day 2, respectively. Mortality started occurring from day 3 ( $5.7 \pm 10.1\%$ , boat control) and 4 ( $9.4 \pm 14.4\%$ , silent control) in the control treatments but with lower mortality than in the airgun array exposed treatment (day 3,  $47.5 \pm 19.2\%$  dead nauplii). In the aliquots from days 5 and 6 after the treatments, almost all of the airgun array exposed nauplii were dead, in contrast to the control groups, in which the proportion of dead nauplii was  $49.2 \pm 12.0\%$  on day 5, and  $72.6 \pm 21.8\%$  day 6 in the silent control and  $4.0 \pm 10.1\%$  on day 5 and  $48.4 \pm 17.9\%$  on day 6 in the boat control.

### 3.3. Growth and development

There was a significant interaction between treatment and time (days) on the body length ( $\mu\text{m}$ ) of the nauplii, with lower growth from day 1–4 in the airgun array exposed treatment than in the control treatments (Fig. 6, Table 3). On day 1 after treatment, the average body length was  $125 \pm 10 \mu\text{m}$ . After 4 days, the average body length in the airgun array exposed nauplii was  $128 \pm 7 \mu\text{m}$ , which differed from the silent control ( $144 \pm 8 \mu\text{m}$ ) and the boat control ( $146 \pm 7 \mu\text{m}$ ). Over the first 4 days after exposure, the growth rates were  $1.7$  ( $4.1\%$  in total),  $5.4$  ( $12.5\%$ ), and  $6.1 \mu\text{m d}^{-1}$  ( $18.7\%$ ) in the airgun array exposed, silent control, and boat control, respectively.

Naupliar development stages (NI–NIV) were positively correlated with body length ( $\mu\text{m}$ ) (Table 3). In all treatments,  $>50\%$  of the nauplii reached stage NII one day after exposure. By day 4,  $43 \pm 26.9\%$  of the airgun array exposed nauplii were still in NI, compared with  $11 \pm 12.7\%$  in the silent control and  $1 \pm 3.6\%$  in the boat control. Only four individuals reached stage NIV: 3 in the silent control and 1 in the boat control (Fig. 6).

## 4. Discussion

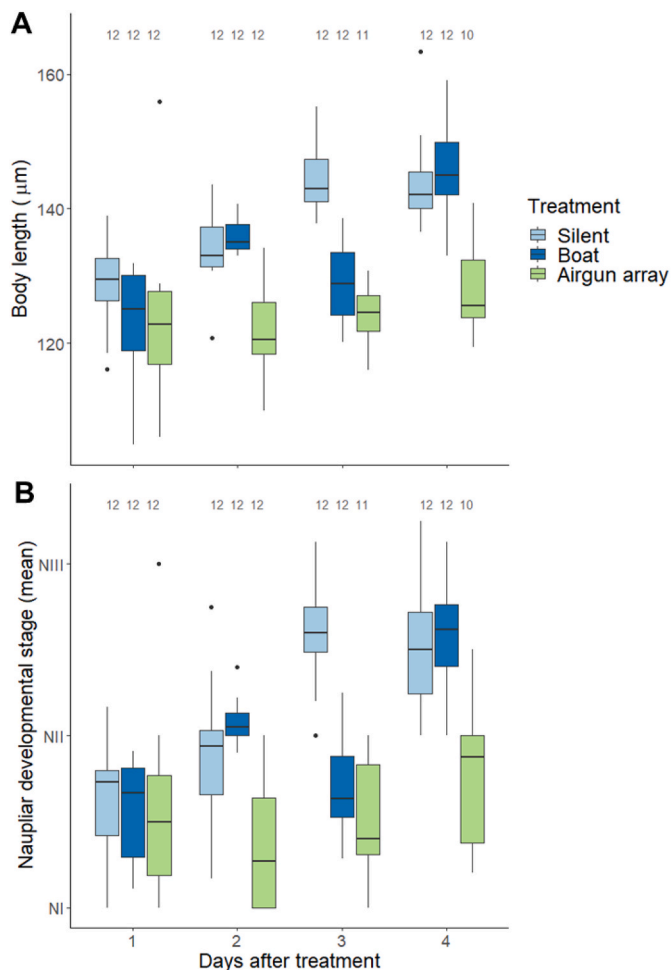
### 4.1. Effects on mortality and development

There were statistically significant effects of airgun discharges on mortality and development of *Acartia* nauplii. This is consistent with previous studies reporting that zooplankton in close proximity to an airgun discharge incurred higher immediate and delayed mortality than unexposed groups (Table 1, references therein). Although we did not test the effects of distance from the airgun, previous studies suggest that the

impact may be limited to relatively close proximity (Fields et al., 2019).

The results observed here are consistent with many previous studies that show small effects of airgun discharges on zooplankton mortality (Table 1). For example, no effects were detected in bivalve larvae sampled 2 km away from the source after exposure to airgun discharges (Parry et al., 2002) or in adult scallops (*Pecten fumatus*) sampled up to 1 km from the source shortly after exposure (Harrington et al., 2010). Similarly, Fields et al. (2019) reported that the mortality of *C. finmarchicus* adults to a two airgun array discharge increased ( $<5\%$ ) compared to that of the control groups, but only at  $< 10$  m from the airguns and no effects at distances from 10 to 50 m (Fields et al., 2019). There are also notable differences in these results from previous studies. For example, in contrast to Fields et al. (2019), this study found significantly higher mortality in the exposed animals compared to the controls at distances of 50–1200 m. Although the sound exposure levels were higher in Fields et al. (2019) than those in this study, the animals in this study were exposed to multiple airgun discharges that resulted in a cumulative exposure that lasted much longer (Table 2). The cumulative exposure of multiple blasts coupled with the younger stage used in this study may help to explain the higher mortality. Despite the higher mortality, the immediate mortality observed in this study is much lower than the 50% mortality in zooplankton at  $> 1$  km from the source (McCauley et al., 2017). Even though the absolute immediate mortality was lower than that reported by McCauley et al. (2017), the relative increase in mortality compared to the controls was somewhat greater in this study ( $>$  threefold increase) than in McCauley et al. (2017) (two- to threefold increase). However, in McCauley et al. (2017), the mortality in the controls was  $\sim 20\%$  compared to less than 4% in this study. In this study, the mortality rate in nauplii directly after exposure was lower than the natural mortality rates observed in *Acartia* nauplii (up to  $0.35 \text{ d}^{-1}$ ), although this is dependent on temperature, season, and region (Elliott and Tang, 2011). This indicates that the population-level effect of airgun exposure might not be detectable from the background mortality.

Studies on delayed mortality in zooplankton after airgun discharges are scarce. Day et al. (2017) reported increased mortality in scallops 14 days after exposure to airgun discharges, but the effect vanished after 4 months. No effects were reported in scallops up to 10 months after a seismic survey (Przeslawski et al., 2018), rock lobsters (*Jasus edwardsii*) the following weeks to years after exposure (Parry and Gason, 2006), American lobsters (*Homarus americanus*) several months after exposure



**Fig. 6.** The body length ( $\mu\text{m}$ ) and developmental stage (NI–NIV) of *Acartia tonsa* nauplii after experimental exposure to a silent control, a boat control, or an airgun array from 1 to 4 days after exposure. A) Body length ( $\mu\text{m}$ ) of the nauplii and B) naupliar developmental stage (NI–NIV), calculated as the mean proportion of stages for every aliquot. The total number of aliquots counted per treatment per day is noted at the top of the figure. The horizontal lines indicate the median, and boxes indicate the 25th percentile (lower quartile), and the 75th percentile (upper quartile).

(Payne et al., 2007), or zoea larvae of Dungeness crab weeks after exposure (Pearson et al., 1994). Fields et al. (2019) appear to be the only study to investigate delayed mortality (up to seven days after exposure) from airgun discharges in copepods. They found nominally (9%) higher mortality in exposed copepods, but only at a distance of <10 m from the source, in contrast to this study, in which elevated mortality was observed one day after exposure.

The airgun array exposed nauplii grew less and developed slower over four days than the boat and silent control groups. The slower development in the airgun array treatment nauplii was correlated with decreased growth. These results are consistent with the developmental delays and morphological abnormalities observed in scallop larvae after exposure to sounds recorded from an airgun array (de Soto et al., 2013). Developmental delays may also manifest at later life stages after exposure to various stressors during the early developmental stages and impact survival or fitness in later life stages (Gebauer et al., 1999; Pechenik, 2006). For example, gastropod larvae (*Crepidatella dilatata*) exhibited delayed development and decreased fitness after exposure to hypoxic conditions (Segura et al., 2014), and barnacles (*Balanus glandula*) grew slower after exposure to low food concentrations (Emlet and Sadro, 2006). The progression through developmental stages and increase in body length observed in the control groups in our study is more

similar to the development naturally observed in *Acartia tonsa* nauplii cultured in 10–15 °C water than is the development in the airgun exposed nauplii (Leandro et al., 2006). Slowed or arrested development at naupliar stages can reduce fitness or cause death (Gebauer et al., 1999). Thus, mortality could be affected long after seismic exposure. The population-level effects that this might have are uncertain.

#### 4.2. The challenges of upscaling these results to a real-life seismic survey

Compared to a large seismic survey, the size and the number of airguns were much smaller in this study. Typically, 18–48 airguns are distributed in several subarrays during standard seismic surveys. The total chamber volume of an airgun array usually ranges from 1220 to 5300 inch<sup>3</sup>, covering survey areas of 1000–3000 km<sup>2</sup> (Hovem and Tronstad, 2012; Slabbekoorn et al., 2019). The source level of an airgun array may reach up to 260 dB re  $\mu\text{Pa}$  at 1 m (Hildebrand, 2009). The modeled sound exposure level of such airgun arrays is 200 dB re 1  $\mu\text{Pa}^2\text{s}$  near the source (Handegard et al., 2013), which is significantly higher than that in this study. The SEL generated at approximately 1 km by a large seismic array is equivalent to that measured at the closest distance in this study according to Handegard et al. (2013). However, since several properties of the sound will change with distance (Erbe et al., 2016), additional field studies such as those of McCauley et al. (2017) are warranted. Nonetheless, small-scale experimental studies such as this one provide a level of experimental control over both the exposure and the previous experience of the animals that is impossible to achieve in a field study.

Zooplankton will be exposed to airgun discharges when a seismic survey is conducted but over varying time durations and sound levels. In addition, seasonal, diel, and species-specific changes in copepod distribution, both within and among species (Hygum et al., 2000; Thor et al., 2005), can influence exposure level and, therefore, the impact of seismic discharges. The total duration of the exposure in this study was short compared to a real seismic survey. Seismic activity can run over several months, shooting both day and night. Our exposure time lasted ~2.5 h – i.e. individual zooplankton may not be exposed for much longer than a few hours during a survey, although this will depend on factors such as survey design and water current.

The areas where these surveys are conducted only comprise a small fraction of the areas where zooplankton are distributed. For example, on the Norwegian Continental Shelf, 16740 km<sup>2</sup> was covered with 3D/4D/4C seismic surveys in 2020 (NDP, 2021). In comparison, the Norwegian Continental Shelf comprises more than 2 million km<sup>2</sup>. Thus, regional population-level effects from a minute excess mortality (~14% vs. ~4% mortality in the airgun and controls, respectively) in these areas seem unlikely. A model accounting for parameters such as ocean currents, seasonality, survey duration, area coverage, and vertical migration is needed to assess the potential population effects from seismic surveys on zooplankton.

## 5. Conclusions

The results of this study suggest that airgun array discharges affect the growth and mortality of *Acartia* in early naupliar stages. However, the degree of impact is likely to be stage- and species-specific and may be difficult to separate from background mortality. The growing demand for subsea minerals is driving exploration, which will ensure the continued use of seismic surveys in the coming decades. Understanding the potential impacts on key species and specific life stages is needed to inform the spatial locations and extent, and the seasonal timing, of these surveys.

#### Credit author statement

Emilie Hernes Vereide: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Writing - Original Draft,

Writing - Review & Editing, Visualization Project administration, Marina Mihaljevic: Investigation, Howard Browman: Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing, Supervision, Funding acquisition, David M. Fields: Conceptualization, Methodology, Writing - Original Draft, Writing - Review & Editing, Funding acquisition, Mette Dalgaard Agersted: Josefin Titelman: Karen de Jong: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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