Effects of Pile-driving Playbacks and Cadmium Co-Exposure on the Early-Life-Stage Development of the Norway Lobster, *Nephrops norvegicus*

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Permits and Ethical Approval

The research conducted falls outside the remit of the Animals (Scientific Protection) Act 1986, therefore required no licensing or ethical approval. Research was conducted under best practices, and in accordance with Edinburgh Napier University's ethical guidelines.

No specific chemical licensing was required. Prior to the study, biosecurity and chemical handling procedures were developed in conjunction with St Abbs Marine Station, where the laboratory experiment was performed, and in consultation with both the Scottish Environmental Protection Agency (SEPA) and Marine Scotland to ensure negligible risk of accidental release of Cd into the broader environment.

Methods

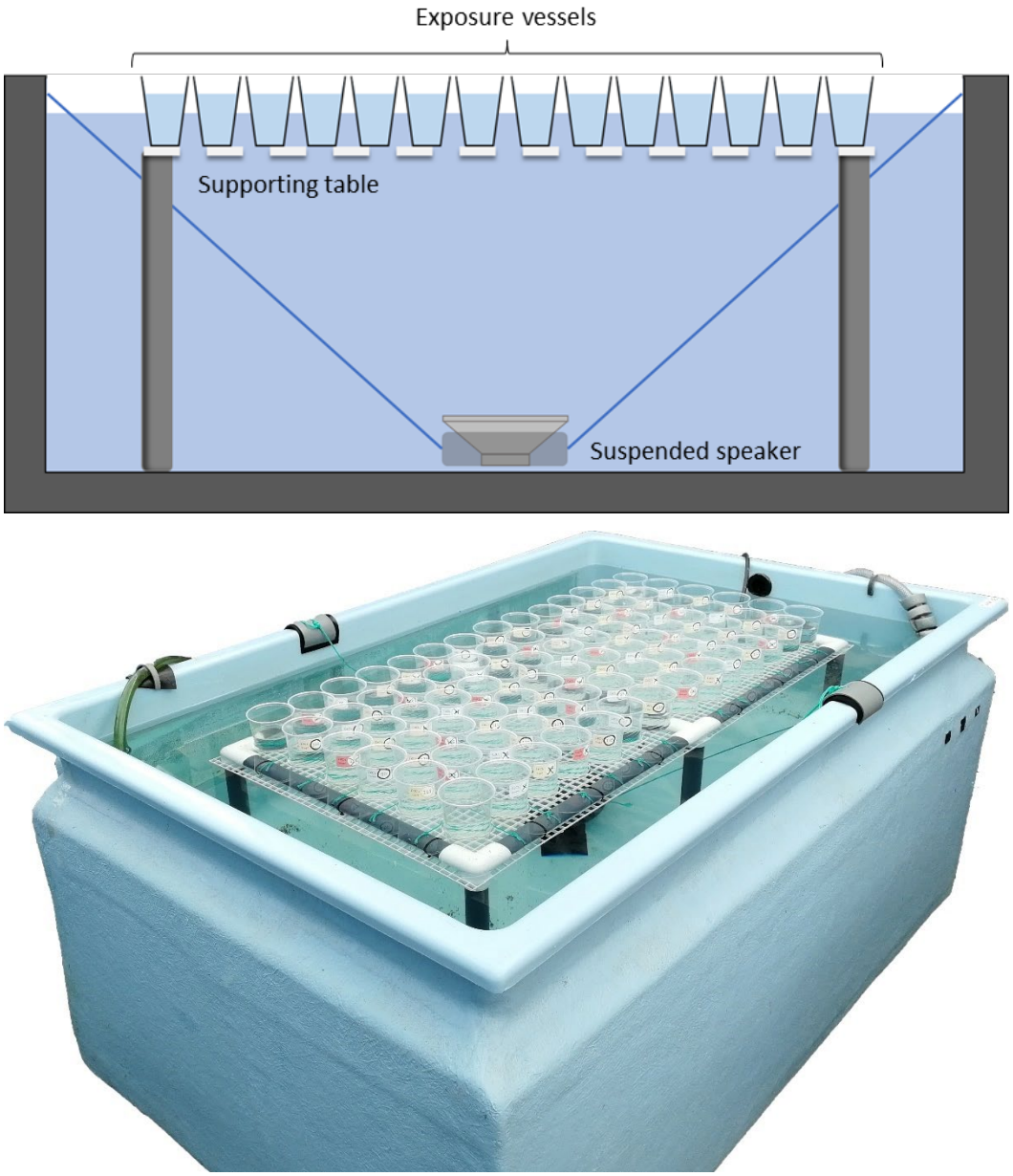

Figure S1: Top: Longitudinal cross-sectional schematic of the exposure systems. Bottom: Photograph showing exposure vessels within an exposure system.

Chemical exposures

Dosing solutions

Cadmium dosing solutions were made from a 1 $g_{[Cd]}$ L^{-1} primary stock solution of cadmium chloride (Sigma Aldrich, CAS# 654054-66-7, 99.995%) prepared in deionised

(DI) water. Dosing solutions were subsequently created from the primary stock via serial dilution with DI water.

Concentrations of dosing solutions and dilution factors varied between experiments according to required working volumes. For Experiment 1, where working volumes were lower, dosing solutions of concentrations of 0 $\mu g_{[Cd]}\ L^{-1}$, 250 $\mu g_{[Cd]}\ L^{-1}$, 2500 $\mu g_{[Cd]}\ L^{-1}$, 25000 $\mu g_{[Cd]}\ L^{-1}$ were created. For Experiment 2, dosing solutions of concentrations of 0 $\mu g_{[Cd]}\ L^{-1}$, 800 $\mu g_{[Cd]}\ L^{-1}$, 8000 $\mu g_{[Cd]}\ L^{-1}$, 80000 $\mu g_{[Cd]}\ L^{-1}$ were utilised (Table S1).

Table S1: Details of the dilution series for creation of cadmium dosing solutions

	To Create:	Stock to Use	Quantity of Stock	Volume of deionised water (ml)	Dosing Stock Cd^{2+} concentration
Experiment 1					
	Primary stock	$CdCl_2$	0.082 g	50.00	1 g L^{-1}
	$High_{[Cd]}$ stock	Primary Stock	0.833 ml	32.50	25000 µg L^{-1}
	$Medium_{[Cd]}$ stock	$High_{[Cd]}$ stock	3.30 ml	29.70	2500 µg L^{-1}
	$Low_{[Cd]}$ stock	$Medium_{[Cd]}$ stock	3.00 ml	27.00	250 µg L^{-1}
Experiment 2					
	Primary stock	$CdCl_2$	0.082 g	50.00	1 g L^{-1}
	$High_{[Cd]}$ stock	Primary Stock	4.000 ml	46.00	80000 µg L^{-1}
	$Medium_{[Cd]}$ stock	$High_{[Cd]}$ stock	5.00 ml	45.00	8000 µg L^{-1}
	$Low_{[Cd]}$ stock	$Medium_{[Cd]}$ stock	5.00 ml	45.00	800 µg L^{-1}

Dosing dynamics

Chemical exposures were conducted under semi-static renewal conditions, with 95% water changes and full cadmium renewal each water change. Water changes occurred twice weekly during Experiment 1 and daily during Experiment 2.

At each dosing interval, one millilitre of dosing solution was added to 249ml (Experiment 1) and 799 ml (Experiment 2) of UV sterilised seawater, resulting in final working volumes of desired nominal concentrations (detailed in section 0). Where mortalities occurred in individual replicates, both dosing and dilution volumes were adjusted proportionately to maintain comparable stocking densities and exposure concentrations.

In all cases, dosing solutions were diluted into working solutions in the absence of larvae to prevent exposure to excessive cadmium concentrations. The large dilution factors from dosing stocks to working solutions were similarly chosen to increase likelihood of achieving near-nominal cadmium concentrations, as well as to limit the undesirable reduction of salinity in the exposure vessels.

Replication and allocation

In each study, replication was achieved using conspecific larvae originating from a single berried female. Twenty newly hatched larvae (< 24 hours old) were randomly allocated to each treat treatment.

Experiment 1: Phenomenological effects

A total of 160 ZI larvae were utilised. Larvae were evenly distributed between treatment groups resulting in n=20 independent replicates per treatment. Due to timing of hatching, larvae were allocated over a two-day period, with 80 larvae allocated on each of the two days.

Larvae were maintained individually in 330 ml BPA-free, food-grade virgin polypropylene plastic cups containing 250 ml of UV sterilised seawater arranged with the exposure system according to their assigned cadmium concentration in a 14 x 6 Latin-square array to account for environmental factors and sound gradient effects (Figure S2).

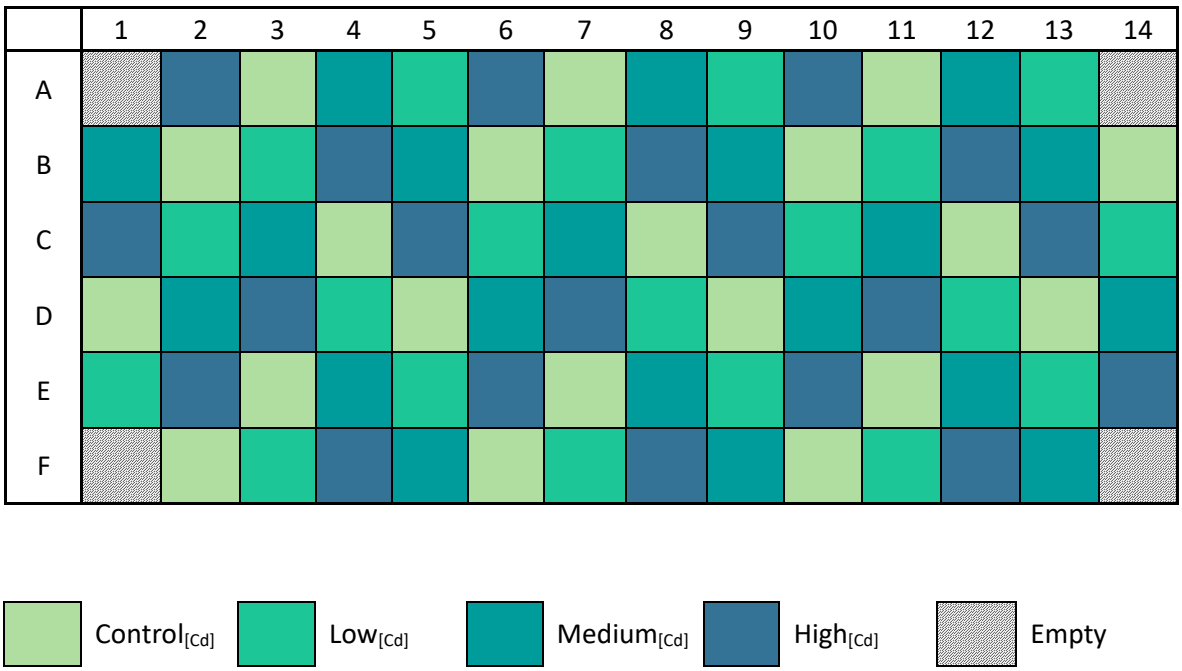

Figure S2: Schematic showing Latin-square arrangement of cadmium treatments within the exposure systems. *Alphanumeric indices refer to the equally spaced positions on the acoustically transparent table as shown in Figure S1*

Experiment 2: Mechanistic effects

A total of 672 larvae were utilised for this study. Exposures were undertaken in 1000 ml borosilicate glass beakers, containing a maximum of 800 ml of UV sterilised seawater of a stated nominal cadmium concentration. To provision sufficient tissue quantities to enable biomarker analyses, 12 larvae were allocated to each exposure vessel, with each exposure vessel being regarded as a single independent replicate. Over the course of 12 days, a total of seven replicates was set up for each treatment group.

Exposure vessels were randomly allocated to one of 16 positions within the central portion of the exposure system where sound levels were most consistent (Figure S3).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A					**1**		**5**	**9**		**13**				
B					**2**		**6**	**10**		**14**				
C					**3**		**7**	**11**		**15**				
D					**4**		**8**	**12**		**16**				
E														
F														

Figure S3: Schematic showing available positions of cadmium treatments within the exposure systems. *Alphanumeric indices refer to the equally spaced positions on the acoustically transparent table as shown in Figure S1.*

Tissue homogenisation for oxidative stress assays

Replicate whole-organism samples were homogenised in 800 µl Tris-HCl (50 mM, 0.15 M KCl, pH 7.4) buffer solution using a motorised pestle, and spun at 10,000 RPM for three minutes in an Eppendorf Mini Spin centrifuge. The resulting supernatant was split into aliquots of sufficient volume for each of the oxidative stress assays, and these aliquots re-frozen at -80 °C until required. This splitting and aliquoting step minimised requirement for repeated freezing/thawing of the samples and assisted with standardisation of each tissue sample between assays. Quantitative assays were corrected for protein content as determined by Bradford assay (see section XXXX).

Oxidative stress assays were conducted in 96 well plates using the colorimetric methods described below. In all instances, samples were plated and absorbance read in triplicate using a Spectramax M5 Multi-Mode Microplate Reader. Where required, standards, blanks, and positive controls were likewise conducted in triplicate within the same plate as samples for consistency and robustness.

Superoxide Dismutase (SOD) inhibition

Superoxide Dismutase inhibition was quantified using a Sigma-Aldrich SOD Determination Kit (19160). Each 20 µl of sample homogenate was combined with 200 µl of WST Working solution and 20 µl of Enzyme Working solution. In addition, three blanks were also created. Blank 1 replaced tissue homogenate with ultrapure (Milli-Q) water. Blank 2 replaced the Enzyme Working solution with Dilution buffer. Blank 3 replaced both the tissue homogenate and dilution buffer. Plates were incubated at 37

°C for 20 minutes prior to being read at 450 nm on the plate reader. SOD inhibition was then calculated using the equation:

$$\text{SOD inhibition rate } (\%) = \frac{(A_{\text{Blank 1}} - A_{\text{Blank 3}}) - (A_{\text{Sample}} - A_{\text{Blank 2}})}{(A_{\text{Blank 1}} - A_{\text{Blank 3}})} \times 100$$

Catalase (CAT)

Catalase activity was quantified using a Cayman Chemical Catalase Assay Kit (707002), utilising the peroxidatic conversion of methanol to formaldehyde. Firstly, 20 µl of sample homogenate was combined with 100 µl of diluted Assay Buffer, 30 µl of methanol, and the catalytic reaction initiated by addition of 20 µl of diluted hydrogen peroxide. Plates were then covered and incubated at room temperature on a plate shaker for 20 minutes before the reaction was terminated by addition of 30 µl of potassium hydroxide. Colour was developed by addition of 30 µl of Catalase Purpald, a further 10-minute room-temperature incubation on the plate shaker, addition of 10 µl of catalase potassium periodate, and a final five-minute incubation on the plate shaker at room temperature. Absorbance was then read at 540 nm, and samples compared to a range of formaldehyde standards and a catalase positive control developed using the same method.

Glutathione (GSH)

Glutathione concentration was determined according to methods outlined by Smith et al. (2007) adapted from (Owens and Belcher, 1965). Each 20 µl homogenate sample was combined with 20 µl of 10 mM 5,5-dithiobis-2-nitrobenzoic acid) (DNTB), 260 µl Tris-HCl (50 mM, 0.15 M KCl, pH 7.4) buffer, and 20 µl of 2U ml^{-1} glutathione reductase (GR). Reaction was initiated by addition of 20 µl NADPH, samples incubated at room temperature for six minutes, and absorbance read at 412 nm against a 125-1000 µM GSH standard range.

Glutathione Peroxidase (GPx)

Glutathione peroxidase was quantified using Cayman Chemical Glutathione Peroxidase Assay Kit (703102). The assay indirectly measures GPx activity through a coupled reaction with glutathione reductase (GR), whereby glutathione is oxidised and subsequently recycled to a reduced state by GPx and GR respectively. Each 20 µl homogenate sample was combined with 50 µl of Assay Buffer, 50 µl of Co-Substrate

Mixture, and 50 µl NADPH. Redox reactions were initiated by addition of 20 µl of cumene hydroperoxidase, and the plate absorbance immediately read at 340 nm. Further absorbance readings at 340 nm were then taken at one-minute intervals for five minutes to produce activity curves. Sample absorbance readings were corrected for background non-enzyme related absorbance using background wells which replaced sample homogenate with additional Assay Buffer. GPx activity was then calculated using the following equations between two time-points along the linear proportion of the absorbance curves:

$$\Delta A_{340}/\text{min} = \frac{A_{340}(\text{Time 2}) - A_{340}(\text{Time 1})}{\text{Time 2(min)} - \text{Time 1(min)}} \qquad \text{GPx activity} = \frac{\Delta A_{340}/\text{min}}{0.00373\ \mu M^{-1}} \times \frac{0.19\ \text{ml}}{0.02\ \text{ml}}$$

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) were quantified using a method derived from those described by from those described by (Bouskill et al., 2006; Camejo et al., 1998; Smith et al., 2007). Here, 40 µl tissue homogenate, 10 µl of 1M butylated hydroxytoluene (BHT) in ethanol, 140 µl of 1mM ethylenediaminetetraacetic acid phosphate buffered saline (EDTA PBS) at pH 7.4, 50 µl of 50% (w/v) trichloroacetic acid (TCA), and 75 µl of 1.3% (w/v) thiobarbituric acid (TBA) in 0.3% (w/v) sodium hydroxide (NaOH) were combined. Plates were then incubated at 60 °C for one hour, and absorbance read at 530 nm and 630 nm wavelengths, and absorbance calculated as, $\Delta A_{\text{TBARS}} = A_{530} - A_{630}$. Samples were then compared against a 0.5-25 nM 1,1,3,3-tetraethoxypropane (TEP) in ethanol standard range.

Protein quantification (Bradford assay)

Sample protein was quantified using the method outlined by (Bradford, 1976). Sample homogenates were diluted 1:10 using Tris-HCl buffer, and in quadruplicate, 10 µl of diluted homogenate combined with 290 µl of Bradford reagent. Plates were incubated at room temperature for five minutes, and absorbance read at 595 nm. Samples were then compared against a 0-1000 µg L^{-1} bovine serum albumin (BSA) standard range.

Metallothionein (MT)

Metallothioenein was quantified in accordance with the methods derived from (Viarengo et al., 1997) and (Cenov et al., 2018), excepting the homogenisation buffer which was as described in Section 0 with the addition of 0.01% v/v 2-mercaptoethanol

as a reducing agent. The tissue homogenate was further centrifuged at 20,000 x g for 20 minutes, and 200 µl of supernatant extracted. To the supernatant, 210 µl of cold (-20 °C) absolute ethanol and 16 µl of chloroform were added, and the samples centrifuged cold (0-4 °C) at 6000 x g for 10 minutes. The supernatant was extracted, and three volumes of cold (-20 °C) absolute ethanol added, and the solution left to precipitate at -20 °C for one hour, before being centrifuged at 6000 x g for 10 minutes. The resulting pellets were washed using ethanol:chloroform homogenization buffer (87:1:12), centrifuged again at 6000 x g for 10 minutes, and the pellets dried under a nitrogen gas stream to complete evaporation. Dried pellets were resuspended in 300 µl of 5mM Tris-HCl, 1 mM EDTA, pH 7, and 20 µl of the resuspended metallothionein fraction combined with 280 µl of 0.43mM DNTB buffered to pH 8 using 0.2 M phosphate buffer. Samples were incubated at room temperature for 30 minutes, and absorbance read at 412 nm against a 0-1000 µM GSH standard range assuming 18 Cys residues per metallothionein residue (Cenov et al., 2018; Zhu et al., 1994).

Results

Sound exposures

A)

Ambient sound treatment: RMS SPL (dB re: 1 µPa)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	117.6	117.3	118.1	117.8	118.1	118.1	118.4	117.9	118.4	118.4	118.1	117.8	117.8	117.4
B	118.4	118.0	118.2	118.2	118.0	118.4	118.5	118.5	118.5	118.5	117.6	117.9	118.0	117.5
C	118.2	118.2	118.1	118.0	118.1	117.9	118.5	118.6	117.6	118.5	117.9	117.8	117.8	117.6
D	118.1	117.9	118.1	118.2	118.0	118.1	117.4	118.5	118.7	118.3	117.7	117.8	117.9	117.7
E	118.0	117.8	117.8	118.0	117.5	118.2	118.4	118.3	118.7	118.0	118.0	117.8	117.7	117.7
F	118.1	117.8	117.5	118.0	118.1	118.2	118.3	118.3	118.6	118.0	117.8	117.9	117.7	117.2

Piling sound treatment - ambient phase: RMS SPL (dB re: 1 µPa)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	117.8	117.9	118.0	118.1	118.8	118.1	118.1	117.7	117.8	118.0	118.3	118.0	118.4	118.0
B	118.0	118.1	118.0	118.2	118.4	118.3	117.8	117.7	118.0	118.1	118.1	118.1	118.4	118.0
C	117.9	118.2	118.0	118.0	118.3	118.6	117.6	117.7	118.2	117.9	118.1	118.4	118.4	118.1
D	117.8	118.1	118.1	118.1	118.2	118.3	117.7	118.0	118.2	117.4	118.2	118.5	118.2	118.2
E	117.8	118.3	118.1	118.2	118.4	118.2	117.7	118.1	118.1	118.0	118.0	118.3	118.2	118.0
F	117.8	118.1	118.0	118.5	118.1	118.1	117.8	118.1	117.8	118.0	118.0	118.4	118.1	117.9

B)

Piling sound treatment - piling phase: Peak-Peak SPL (dB re: 1 µPa)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	167.2	166.1	166.3	169.8	169.0	171.9	170.9	171.7	170.2	168.4	169.7	166.4	167.1	167.7
B	167.0	168.2	168.7	169.7	171.2	172.1	170.7	171.3	171.4	167.7	169.3	168.3	167.2	167.6
C	166.8	165.4	169.3	170.8	171.7	171.3	171.4	171.4	171.2	169.6	169.6	167.4	166.6	166.4
D	165.6	167.8	168.6	169.8	171.7	170.9	171.8	171.8	170.4	170.9	170.8	169.6	168.1	166.4
E	166.9	167.0	168.5	168.6	170.5	170.6	171.8	171.8	169.9	170.8	168.0	168.0	169.1	167.0
F	166.7	169.1	166.3	169.3	170.4	170.9	170.7	171.3	170.6	170.2	169.3	167.7	168.4	168.3

Piling sound treatment - piling phase: RMS SPL (dB re: 1 µPa)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	135.7	133.4	134.9	136.6	136.9	139.5	138.9	140.3	137.1	135.9	136.5	133.8	135.1	135.7
B	134.7	135.5	136.4	137.0	138.7	139.9	137.5	138.6	138.3	134.7	136.8	136.2	134.7	135.5
C	134.7	133.0	136.9	138.6	139.0	139.0	138.2	138.2	138.7	136.5	137.1	135.2	134.7	134.8
D	133.5	135.2	136.2	137.9	139.5	138.0	139.1	139.5	138.2	138.3	138.9	136.6	135.9	134.8
E	135.1	134.2	136.1	136.1	137.4	138.6	139.0	139.4	136.5	137.3	135.2	136.0	136.2	135.1
F	134.8	135.8	134.8	136.7	137.7	138.4	138.2	138.7	137.8	137.8	136.5	135.0	136.4	136.7

Figure S4: Experiment 1 sound pressure measurements. *Heatmap of received sound pressure levels within each exposure vessel as located in the exposure system during A) ambient-playback; B) piling-playback. All measurements are absolute values taken at each location. Alphanumeric indices refer to the equally spaced positions on the acoustically transparent table as shown in Figure S1.*

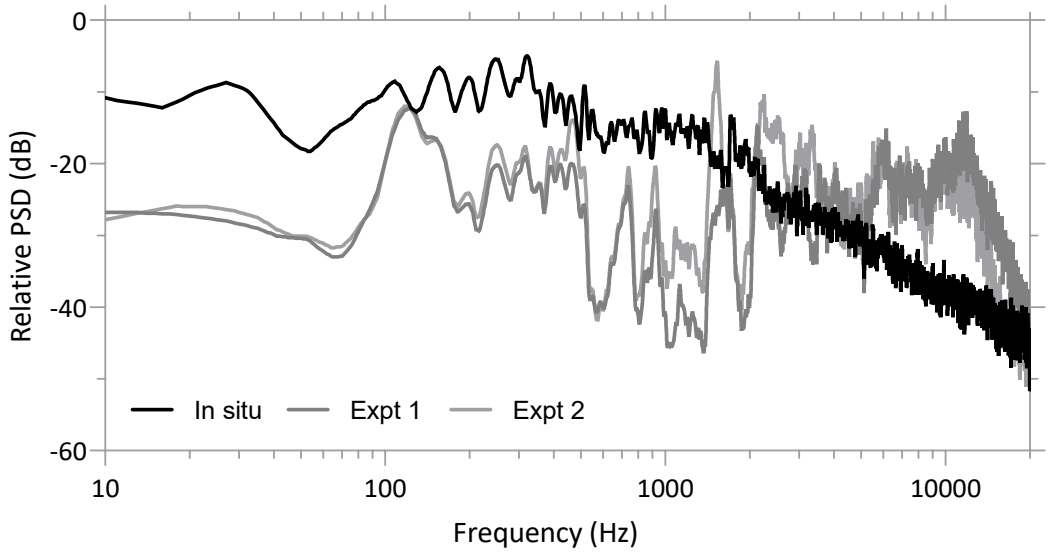

Figure S5: Relative RMS power spectral density (0.1 second Hann window, 50-percent overlap) of piling as recorded in situ and as received in exposure vessels via playbacks in each experiment.

188

189 *Mortality*

190 **Total Mortality**

Table S2: Statistical summary of logistic regression model of overall N. norvegicus mortality. Bold values signify statistical significance p < 0.05

	Estimate	SE	Z value	*p*
(Intercept)	-0.162	0.242	-0.668	0.504
Cadmium	0.474	0.218	2.173	**0.030**
Sound	-0.764	0.398	-1.923	0.055
Sound x cadmium	0.773	0.363	2.130	**0.033**

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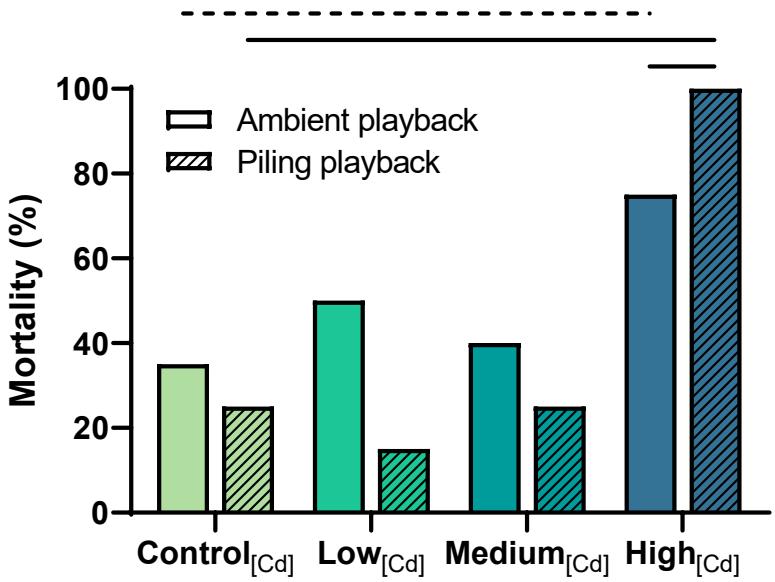

Figure S6: Total N. norvegicus larval mortality. Solid and hatched bars represent ambient- and piling-playback sound treatments respectively. Control$_{[Cd]}$, Low$_{[Cd]}$, Medium$_{[Cd]}$, and High$_{[Cd]}$ represent Cd^{2+} ion concentrations of 0.08 µg L^{-1}, 0.71 µg L^{-1}, 6.48 µg L^{-1}, 63.52 µg L^{-1} respectively Horizontal markers above bars denote significant differences between groups (Fisher's exact test, dashed line uncorrected p <0.05, solid line corrected p <0.05).

192

193 **Temporal patterns of mortality**

194 ***Table S3: Statistical summary of N. norvegicus larval mortality curves.*** *Post-hoc log-rank Mantel-Cox*
195 *comparisons. Uncorrected p values represent those from pairwise comparison. Corrected p values are*
196 *modified values controlling for false discovery rate using Benjamini-Hochberg procedure (α = 0.05).*
197 *Bold values signify statistical significance p < 0.05*

Contrast treatments		df	Z	Uncorrected *p* value	Corrected *p* value
Ambient - Control$_{[Cd]}$	Ambient - Low$_{[Cd]}$	1	1.0057	0.314	0.454
	Ambient - Medium$_{[Cd]}$	1	0.300	0.765	0.793
	Ambient - High$_{[Cd]}$	1	2.017	**0.043**	0.100
Piling - Control$_{[Cd]}$	Piling - Low$_{[Cd]}$	1	-0.807	0.420	0.534
	Piling - Medium$_{[Cd]}$	1	-0.036	0.971	0.971
	Piling - High$_{[Cd]}$	1	4.464	**<0.001**	**<0.001**
Ambient - Control$_{[Cd]}$	Piling - Control$_{[Cd]}$	1	-0.643	0.521	0.561
Ambient - Low$_{[Cd]}$	Piling - Low$_{[Cd]}$	1	-2.310	**0.020**	0.051
Ambient - Medium$_{[Cd]}$	Piling - Medium$_{[Cd]}$	1	-1.039	0.300	0.454
Ambient - High$_{[Cd]}$	Piling - High$_{[Cd]}$	1	2.631	**0.005**	**0.015**

198 *Developmental duration*

199

Table S4: Statistical summary of N. norvegicus development. *Dunn's test post-hoc analysis of timing of transition to Zoea III (top) and Zoea IV (bottom). Uncorrected p values represent those from pairwise comparison. Corrected p values are modified values controlling for false discovery rate using Benjamini-Hochberg procedure (α = 0.05). Bold values signify statistical significance p < 0.05*

	Contrast treatments		n1, n2	Z	Uncorrected p value	Corrected p value
Transition to Zoea III	Ambient - Control[Cd]	Ambient - Low[Cd]	14, 12	-0.177	0.859	0.891
		Ambient - Medium[Cd]	14, 14	1.794	0.073	0.197
		Ambient - High[Cd]	14, 12	2.616	**0.009**	**0.042**
	Piling - Control[Cd]	Piling - Low[Cd]	16, 18	-1.568	0.117	0.209
		Piling - Medium[Cd]	16, 17	-2.630	**0.009**	**0.042**
		Piling - High[Cd]	16, 5	0.578	0.563	0.717
	Ambient - Control[Cd]	Piling - Control[Cd]	14, 16	1.558	0.119	0.209
	Ambient - Low[Cd]	Piling - Low[Cd]	12, 18	0.272	0.786	0.846
	Ambient - Medium[Cd]	Piling - Medium[Cd]	14, 17	-2.837	**0.005**	**0.042**
	Ambient - High[Cd]	Piling - High[Cd]	12, 5	-0.306	0.760	0.846
Transition to Zoea IV	Ambient - Control[Cd]	Ambient - Low[Cd]	14, 10	0.703	0.482	0.595
		Ambient - Medium[Cd]	14, 12	1.818	0.069	0.212
		Ambient - High[Cd]	14, 5	1.938	0.053	0.212
	Piling - Control[Cd]	Piling - Low[Cd]	15, 17	-1.649	0.099	0.212
		Piling - Medium[Cd]	15, 15	-2.744	**0.006**	0.050
		Piling - High[Cd]	-	-	-	-
	Ambient - Control[Cd]	Piling - Control[Cd]	14, 15	1.678	0.093	0.212
	Ambient - Low[Cd]	Piling - Low[Cd]	10, 17	-0.631	0.528	0.616
	Ambient - Medium[Cd]	Piling - Medium[Cd]	12, 15	-2.823	**0.005**	0.050
	Ambient - High[Cd]	Piling - High[Cd]	-	-	-	-

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202

Behavioural fitness

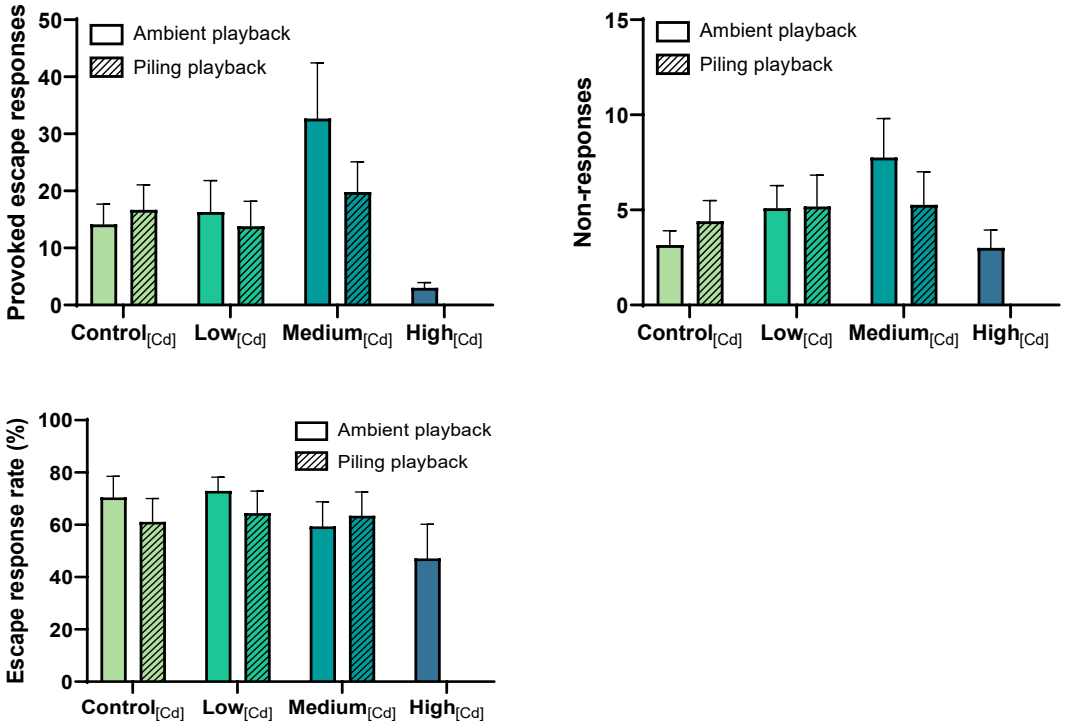

Figure S7: Responses of N. norvegicus juveniles to a simulated threat*. Top-left: number of escape responses provoked. Top-right: number of non-responses to simulated threat. Bottom-left: escape response rate. Solid and hatched bars represent ambient- and piling-playback sound treatments respectively. Control$_{[Cd]}$, Low$_{[Cd]}$, Medium$_{[Cd]}$, and High$_{[Cd]}$ represent Cd^{2+} ion concentrations of 0.08 µg L^{-1}, 0.71 µg L^{-1}, 6.48 µg L^{-1}, 63.52 µg L^{-1} respectively. All bars represent mean values. Error bars represent SE. Absent violins in piling – 100 µg$_{[Cd]}$ L^{-1}treatment consequent of no larvae surviving to metamorphosis)*

Table S5: Statistical summary of N. norvegicus responses to simulated threat. *Comparison by experimental treatment; Kruskal-Wallis test.*

Response	X^2	df	*p*
Total induced escape responses	5.37	6	0.498
Total non-responses	4.55	6	0.602
Response rate (%)	3.65	6	0.725

Table S6: Statistical summary of juvenile N. norvegicus escape responses. Dunn's test post-hoc analysis of principal component scores of escape behaviour dynamics. Uncorrected p values represent those from pairwise comparison. Corrected p values are modified values controlling for false discovery rate using Benjamini-Hochberg procedure (α = 0.05). Bold values signify statistical significance p < 0.05

	Contrast treatments		n1, n2	Z	Uncorrected *p* value	Corrected *p* value
Principle Component 1	Ambient - Control$_{[Cd]}$	Ambient - Low$_{[Cd]}$	679, 623	-0.206	0.837	0.878
		Ambient - Medium$_{[Cd]}$	679, 1076	8.633	**<0.001**	**<0.000**
		Ambient - High$_{[Cd]}$	679, 41	1.470	0.142	0.212
	Piling - Control$_{[Cd]}$	Piling - Low$_{[Cd]}$	782, 768	-1.167	0.243	0.301
		Piling - Medium$_{[Cd]}$	782, 849	-2.385	**0.017**	**0.030**
		Piling - High$_{[Cd]}$	-	-	-	-
	Ambient - Control$_{[Cd]}$	Piling - Control$_{[Cd]}$	679, 782	4.661	**<0.001**	**<0.001**
	Ambient - Low$_{[Cd]}$	Piling - Low$_{[Cd]}$	623, 768	3.648	**<0.001**	**0.001**
	Ambient - Medium$_{[Cd]}$	Piling - Medium$_{[Cd]}$	1076, 849	-6.466	**<0.001**	**<0.001**
	Ambient - High$_{[Cd]}$	Piling - High$_{[Cd]}$	-	-	**-**	**-**
Principle Component 2	Ambient - Control$_{[Cd]}$	Ambient - Low$_{[Cd]}$	679, 623	-1.765	0.078	0.116
		Ambient - Medium$_{[Cd]}$	679, 1076	-15.860	**<0.001**	**<0.001**
		Ambient - High$_{[Cd]}$	679, 41	-2.417	**0.016**	**0.027**
	Piling - Control$_{[Cd]}$	Piling - Low$_{[Cd]}$	782, 768	-1.664	0.096	0.135
		Piling - Medium$_{[Cd]}$	782, 849	-1.317	0.188	0.219
		Piling - High$_{[Cd]}$	-	-	-	-
	Ambient - Control$_{[Cd]}$	Piling - Control$_{[Cd]}$	679, 782	-3.264	**0.001**	**0.003**
	Ambient - Low$_{[Cd]}$	Piling - Low$_{[Cd]}$	623, 768	-2.927	**0.003**	**0.008**
	Ambient - Medium$_{[Cd]}$	Piling - Medium$_{[Cd]}$	1076, 849	11.781	**<0.001**	**<0.001**
	Ambient - High$_{[Cd]}$	Piling - High$_{[Cd]}$	-	-	-	-

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228 *Biometry*

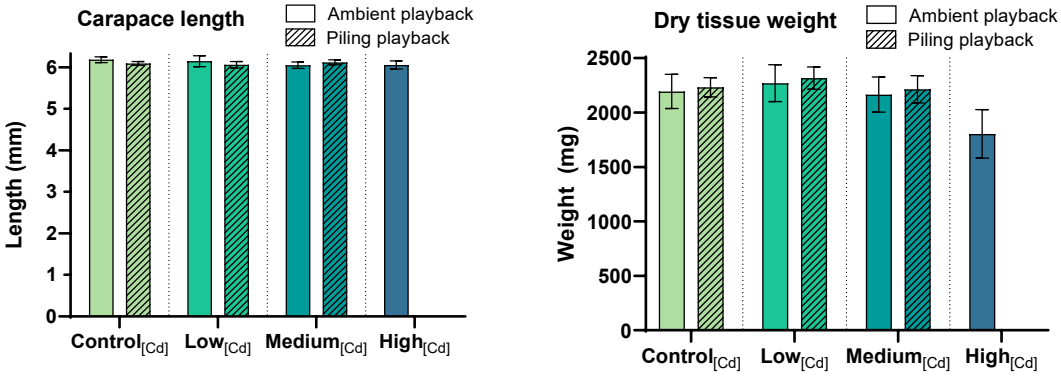

Figure S8: Biometric measurements of N. norvegicus. *Solid and hatched bars represent ambient- and piling playback sound treatments respectively. Control$_{[Cd]}$, Low$_{[Cd]}$, Medium$_{[Cd]}$, and High$_{[Cd]}$ represent Cd^{2+} ion concentrations of 0.08 µg L^{-1}, 0.71 µg L^{-1}, 6.48 µg L^{-1}, 63.52 µg L^{-1} respectively. All bars represent mean values. Error bars represent SE. Absent violin in piling – 100 µg$_{[Cd]}$ L^{-1}treatment consequent of no larvae surviving to metamorphosis.*

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232 ***Table S7: Statistical summary of N. norvegicus oxidative stress biomarker measurements.*** *Top:*
233 *Results of two-way analysis of variance contrasting the effects of sound and cadmium. Bottom: Results of*
234 *Kruskal-Wallis Rank Sums by experimental treatment groups.*

ANOVA

Biomarker	Factor	df	Sum Sq	Mean Sq	F	*p* value
GPx	Sound	1	0.07	0.068	0.011	0.917
	Cadmium	3	40.54	13.513	2.181	0.103
	Sound x cadmium	3	36.59	12.197	1.968	0.132
	Residuals	46	285.05	6.197		
GSH	Sound	1	4808	4808	3.790	0.058
	Cadmium	3	2812	937	0.739	0.534
	Sound x cadmium	3	5777	1926	1.518	0.222
	Residuals	46	58358	1269		
SOD	Sound	1	0.30	0.282	0.019	0.890
	Cadmium	3	56.30	18.755	1.294	0.288
	Sound x cadmium	3	21.00	6.996	0.483	0.696
	Residuals	46	666.70	14.494		

Kruskal-Wallis Rank Sums

Biomarker	χ^2	df	*p* value
CAT	2.8044	7	0.902
TBARS	7.3269	7	0.396
MT	14.565	7	**0.032**

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Table S8: Statistical summary of N.norvegicus metallothionein measurements. *Dunn's test post-hoc analysis. Uncorrected p values represent those from pairwise comparison. Corrected p values are modified values controlling for false discovery rate using Benjamini-Hochberg procedure (α = 0.05). Bold values signify statistical significance p < 0.05*

Contrast treatments		n1, n2	Z	Uncorrected *p* value	Corrected *p* value
Ambient - Control$_{[Cd]}$	Ambient - Low$_{[Cd]}$	7, 6	0.000	1.000	1.000
	Ambient - Medium$_{[Cd]}$	7, 7	1.580	0.114	0.355
	Ambient - High$_{[Cd]}$	7, 7	-0.323	0.747	0.863
Piling - Control$_{[Cd]}$	Piling - Low$_{[Cd]}$	7, 6	-1.137	0.256	0.550
	Piling - Medium$_{[Cd]}$	7, 7	-2.718	**0.007**	0.092
	Piling - High$_{[Cd]}$	7, 7	-0.408	0.683	0.863
Ambient - Control$_{[Cd]}$	Piling - Control$_{[Cd]}$	7, 7	0.629	0.530	0.810
Ambient - Low$_{[Cd]}$	Piling - Low$_{[Cd]}$	6, 6	-0.514	0.607	0.810
Ambient - Medium$_{[Cd]}$	Piling - Medium$_{[Cd]}$	7, 7	-3.669	**<0.001**	**0.007**
Ambient - High$_{[Cd]}$	Piling - High$_{[Cd]}$	7, 7	0.544	0.587	0.810

Table S9: Statistical summary of N. norvegicus oxidative stress biomarker Principal Components analysis. *Results of two-way analysis of variance contrasting the effects of sound and cadmium.*

	Factor	df	Sum Sq	Mean Sq	F	*p* value
Principle Component 1	Sound	1	4.02	1.341	0.583	0.629
	Cadmium	3	1.62	1.618	0.704	0.406
	Sound x cadmium	3	6.12	2.041	0.887	0.455
	Residuals	46	105.8	2.3		
Principle Component 2	Sound	1	2.48	0.826	0.889	0.454
	Cadmium	3	5.67	5.672	6.104	**0.017**
	Sound x cadmium	3	16.25	5.417	5.83	**0.002**
	Residuals	46	42.75	0.929		

Table S10: Statistical summary of N. norvegicus oxidative stress biomarker PC2 post hoc analysis. *Dunn's test. Uncorrected p values represent those from pairwise comparison. Corrected p values are modified values controlling for false discovery rate using Benjamini-Hochberg procedure ($\alpha = 0.05$). Bold values signify statistical significance $p < 0.05$*

Contrast treatments		n1, n2	Z	Uncorrected *p* value	Corrected *p* value
Ambient - Control$_{[Cd]}$	Ambient - Low$_{[Cd]}$	7, 6	0.188	0.851	0.953
	Ambient - Medium$_{[Cd]}$	7, 7	-1.767	0.077	0.240
	Ambient - High$_{[Cd]}$	7, 7	0.781	0.435	0.692
Piling - Control$_{[Cd]}$	Piling - Low$_{[Cd]}$	7, 7	0.892	0.372	0.651
	Piling - Medium$_{[Cd]}$	7, 7	2.361	**0.018**	0.102
	Piling - High$_{[Cd]}$	7, 7	1.002	0.316	0.590
Ambient - Control$_{[Cd]}$	Piling - Control$_{[Cd]}$	7, 7	-0.238	0.812	0.947
Ambient - Low$_{[Cd]}$	Piling - Low$_{[Cd]}$	6, 7	0.459	0.646	0.823
Ambient - Medium$_{[Cd]}$	Piling - Medium$_{[Cd]}$	7, 7	3.890	**<0.001**	**0.003**
Ambient - High$_{[Cd]}$	Piling - High$_{[Cd]}$	7, 7	-0.017	0.986	0.986

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