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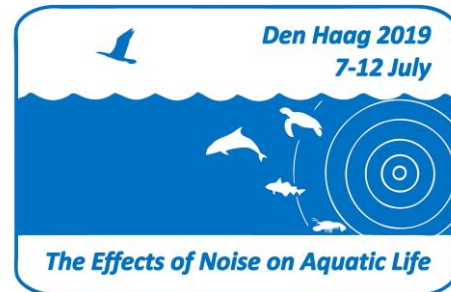
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# Impacts of noise on the behavior and physiology of marine invertebrates: A meta-analysis

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Human-generated noise over the last 60 years has increased concerns regarding the implications for marine species. Many species have been documented to display behavioral and physiological responses to increased noise pollution in our oceans, but the majority of this research has focused on higher trophic organisms. Recently, investigations assessing the impacts of changing soundscapes on entire aquatic ecosystems have begun. To understand the impacts of underwater noise on invertebrate communities, a meta-analysis was conducted on the behavioral and physiological impacts of noise on invertebrates. A systematic review of the literature revealed 1,105 potential studies, which 25 were extracted for data analysis. The studies resulted in 473 data points evaluating the impacts of a plethora of acoustic stimuli on a wide range of marine invertebrate taxa. Here, two acoustic stimuli (ship noise and seismic surveys) were further broken down into behavioral and physiological parameters. Shipping noise had a negative effect size on the behavior and physiology of marine invertebrates. However, seismic surveys resulted in a positive effect size, which was not predicted. While further analysis is required to understand the impacts of these stimuli fully, this meta-analysis reveals the implications that elevated underwater noise levels may have on marine invertebrate communities.

## A. INTRODUCTION

Marine species rely heavily on the use of sound for many biologically relevant activities, including finding a mate (Fine, 1978), defending territories (Maruska and Mensinger, 2009), and prey localization (Au et al., 2004). However, the ocean soundscape is now changing drastically due to human influences (reviewed in Hildebrand, 2009). Shipping traffic has seen a four-fold increase between 1992 and 2012 (Tournadre, 2014), with some areas frequented by almost 20 ships per day, on average (Veirs et al., 2016). In addition to shipping, the number of seismic survey vessels almost doubled between 1992 and 2000 (Schmidt, 2004). These two anthropogenic noise sources are now dominating the lower frequencies (10-500 Hz) in many of our oceans (Nieukirk et al., 2004; Wenz, 1972).

Increased shipping traffic and seismic surveys have elevated ambient noise levels in the world's oceans (reviewed in Hildebrand, 2009). For example, in the Northeastern Pacific Ocean ambient noise levels below 50 Hz have increased 10-12 dB compared to historical levels (2000s vs 1960s), resulting in approximately a 3 dB increase per decade (McDonald et al., 2006). A similar trend has been observed in the Indian Ocean, where ambient noise (5-115 Hz) has increased over 2 dB during the past decade (Miksis-Olds et al., 2013). Even though the increase in ambient noise has not been observed globally (Miksis-Olds and Nichols, 2016), there is still concern about the impact these rising noise levels will have on aquatic species (Popper and Hastings, 2009).

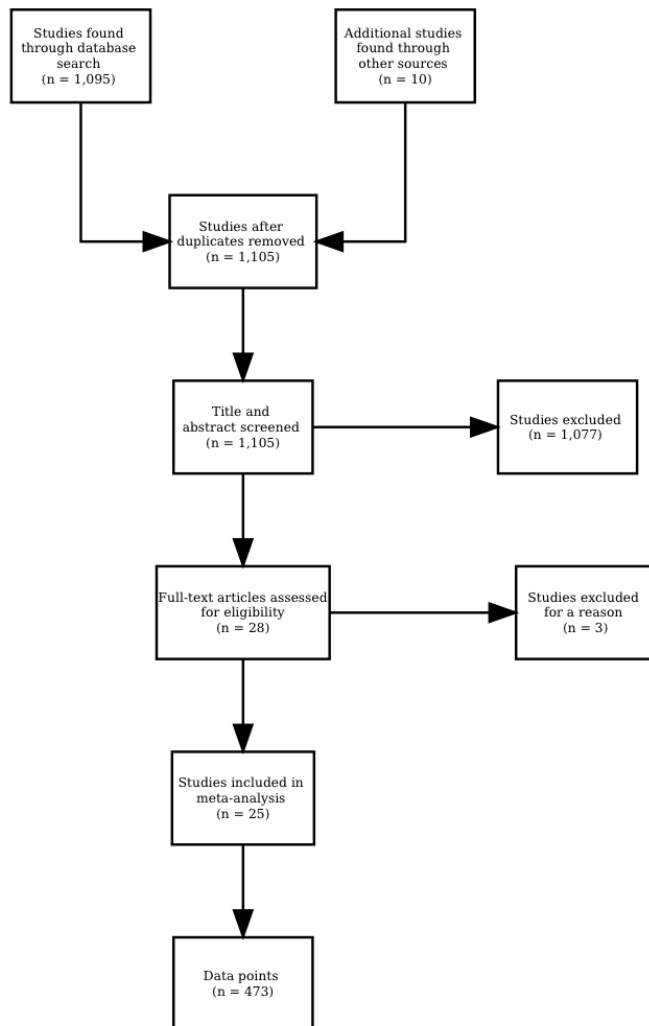
The growing interest in the effects of noise has led to diverse taxa being evaluated (Cox et al., 2018; Gomez et al., 2016). Vessel noise has been shown to alter the foraging behavior of humpback whales (*Megaptera novaeangliae*; Blair et al., 2016) and orcas (*Orcinus orca*; Lusseau et al., 2009), likely influencing their foraging efficiency. A similar trend is observed in fish species, leading to reduced antipredator behaviors (Ferrari et al., 2018; Simpson et al., 2015, 2016) and decreased foraging success (Voellmy et al., 2014) in the presence of anthropogenic noise. Physiological responses have also been documented; for example, chronic stress has been seen in North Atlantic right whales (*Eubalaena glacialis*) due to high shipping traffic (Rolland et al., 2012), and fish have an increase in cortisol in response to noise stimuli, regardless of hearing abilities (Wysocki et al., 2006).

To understand how noise impacts an entire ecosystem, evaluation of invertebrate species for potential impacts is necessary. Research has been conducted on a diversity of taxa, encompassing a wide array of impacts. Spiny lobsters (*Palinurus elephas*) demonstrate both behavioral and physiological changes in the presence of boat noise (Filiciotto et al., 2015), while European green crabs (*Carcinus maenas*) show increased oxygen consumption during noise exposure (Wale et al., 2013b). Cnidarians are also susceptible to sensory damage from noise pollution (Solé et al., 2016). Here, we conducted a meta-analysis on the impacts of anthropogenic noise on the behavior and physiology of invertebrates in order to synthesize and quantify the overall effects of shipping noise and seismic surveys.

## B. MATERIAL AND METHODS

### i. SYSTEMATIC LITERATURE SEARCH

Thompson's Web of Science was used to conduct a systematic literature review, without limitations by publication date or other categories. The specific search terms used were "noise or sound or acoustic\*", "marine or aquatic", and "invertebrate\* or benthic or arthropod\* or cephalopod\* or cnidaria\* or crustacean\* or echinoderm\* or mollus\*". The initial search returned 1,095 potentially relevant peer-reviewed articles. An additional 10 articles were identified through alternate search engines and previous knowledge. The titles and abstracts of the 1,105 studies were reviewed to determine which papers addressed the effects of anthropogenic noise on aquatic invertebrate behavior or physiology (Figure 1). Articles that met these criteria ( $n = 28$ ) were then further evaluated to identify those that met the criteria of original research, listed sound source, experimental control, included mean value with either standard deviation or standard error, and sample size.



**Figure 1: PRISMA diagram demonstrating the screening and selection process for the studies included in this meta-analysis on the effects of noise on marine invertebrates.**

In total, 25 studies from 13 countries met the search criteria. The mean, standard deviation and sample size were extracted from the treatment and control groups of each study. Data were obtained from tables and text whenever possible, and the extraction software GraphClick (Arizona-Software, 2008) was used to collect accurate data from figures when necessary. A total of 473 data points were collected from the 25 studies. Two acoustic stimuli (shipping noise and seismic surveys) were isolated and further analyzed.

## ii. EFFECT SIZE CALCULATION

Effect sizes and variances for each study were calculated using the metafor and MAd packages in RStudio (DeI Re and Hoyt, 2014; R Code Team, 2017; Viechtbauer, 2010). Mean difference (md) was calculated from means from the treatment ( $\bar{Y}_1$ ) and control group ( $\bar{Y}_2$ ) from each study (Eq. 1)

$$md = \bar{Y}_1 - \bar{Y}_2 \quad (1)$$

The standardized mean difference (Hedge's d) was used to determine the overall effect and weight of the studies using their sample sizes ( $n_1$  and  $n_2$ ) and standard deviations ( $s_1$  and  $s_2$ ) using Eq. 2.

$$d = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}} \quad (2)$$

The variance for the Hedge's d value was then calculated using Eq. 3.

$$V_d = \frac{n_1 + n_2}{n_1 n_2} + \frac{d^2}{2(n_1 + n_2)} \quad (3)$$

The directionality of each study was determined to ensure that the negative and positive effect sizes represented the appropriate responses. For example, we ensured that an increase in recruitment (settlement) resulted in a positive effect size since it is a positive response, whereas an increase in heat shock protein resulted in a negative effect size as it is an undesirable response.

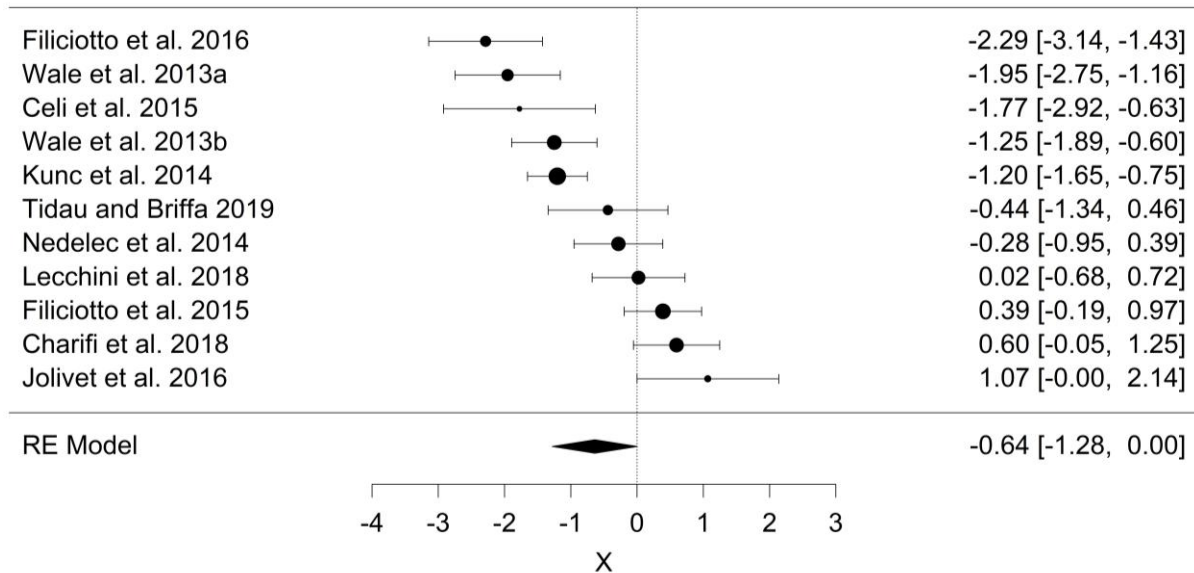
## iii. STATISTICAL ANALYSIS

Statistical analyses were conducted in RStudio using the metafor and MAd packages (Viechtbauer, 2010) to generate forest plots and calculate effect sizes for two of the sound sources, shipping noise and seismic surveys. To understand the impacts of each sound source on behavior and physiology, identical analyses were conducted on each data set. Forest plots summarized effect size and provided confidence intervals for each behavioral and physiological parameter. Boxplots were created to compare the effects sizes of the various taxa included, using the package ggplot2 (Wickham, 2009).

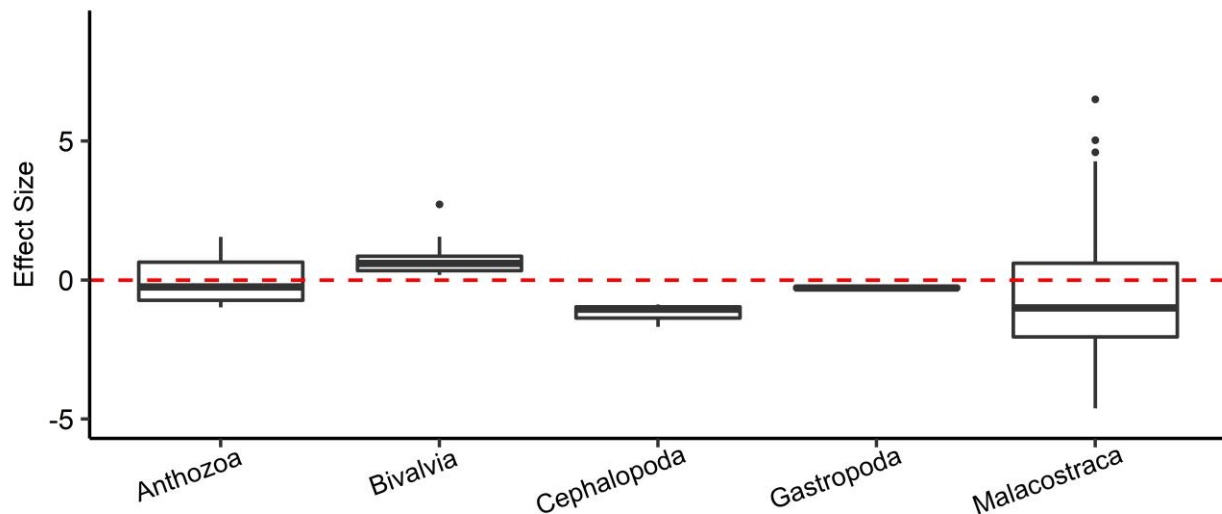
## C. RESULTS

### i. SHIPPING NOISE

We identified 11 studies that evaluated the impacts of shipping noise on marine invertebrates. The forest plot shows an overall negative trend in the physiology and behavior effect sizes of marine invertebrates when exposed to shipping noise (-0.64, CI -1.28, 0.00), with five studies indicating significant results (Figure 2). Five taxa were examined; of these, the classes Bivalvia, Cephalopoda and Gastropoda had significant effect sizes (Figure 3). Cephalopoda and Gastropoda were negative and Bivalvia was positive.



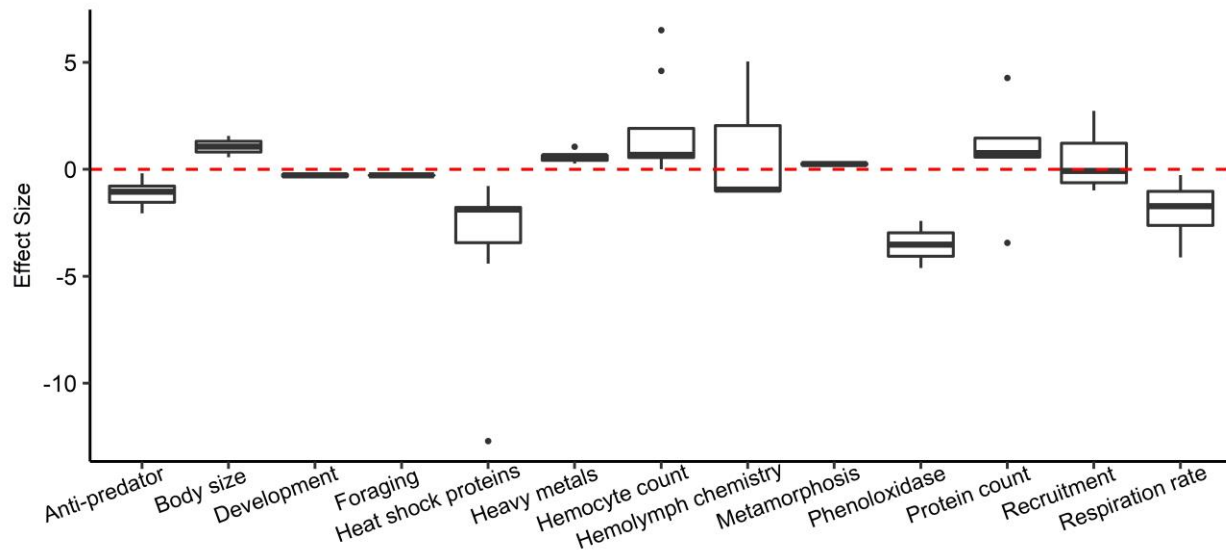
**Figure 2:** Forest plot demonstrating the effect shipping noise has on invertebrate behavior and physiology. Effect sizes with 95% confidence intervals were plotted for each study, along with a summary random effects (RE) model with 95% confidence intervals. Weight of each study in the model is represented by dot size. Vertical dashed line indicates null effect size.



**Figure 3:** Boxplots of effect sizes observed by various invertebrate taxa in response to shipping noise. Horizontal dashed line indicates null effect size.

Impact of shipping noise on marine invertebrates was further broken down into behavioral and physiological parameters (Figure 4). Foraging and antipredator behavior both had a significant negative effect size. However, a wider variety of physiological parameters were examined. Body

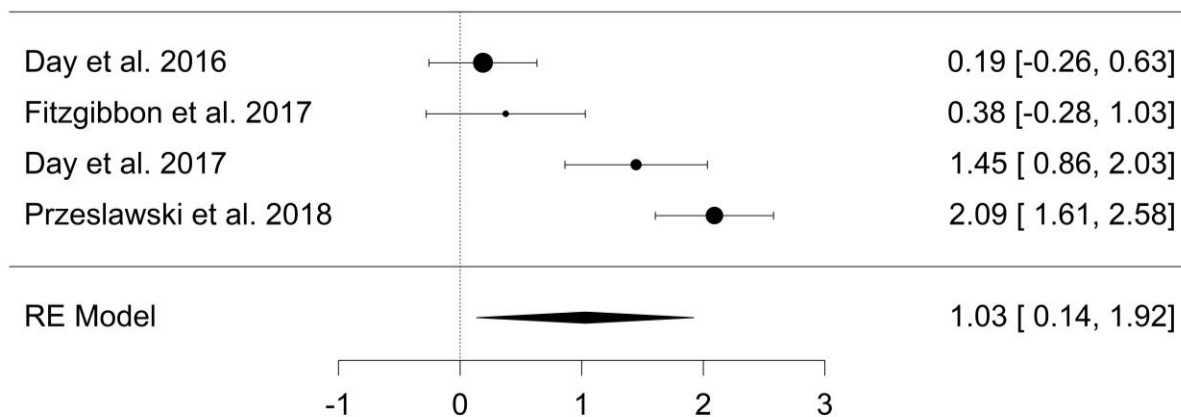
size, development, heat shock protein expression, heavy metal concentration, hemolymph count, metamorphosis, phenoloxidase, and respiration rate all had significant effect sizes. Five physiological parameters had negative effect sizes and four had positive effect sizes.



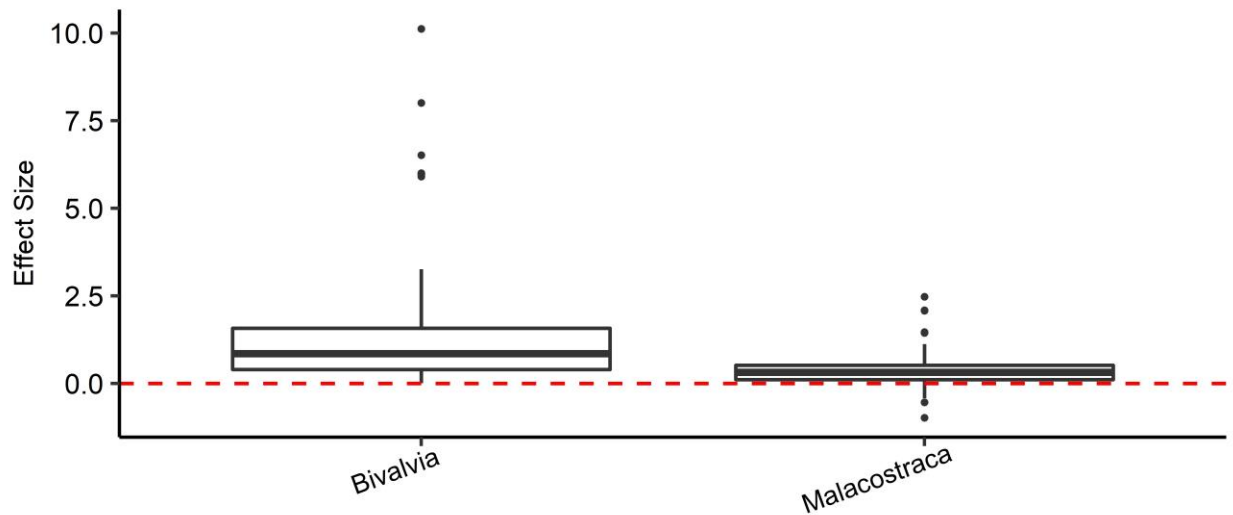
**Figure 4:** Boxplots of the effect sizes observed in marine invertebrates broken down by various behavioral and physiological responses to shipping noise. Horizontal dashed line indicates null effect size.

**ii. SEISMIC SURVEY**

Four studies evaluated the impacts of seismic surveys on marine invertebrates. A forest plot of all studies demonstrated a significant positive trend (1.03, CI 0.14, 1.92). Of the four studies, two were significant and positive (Figure 5). Two taxa were evaluated, and Bivalvia had a significantly positive effect size (Figure 6).

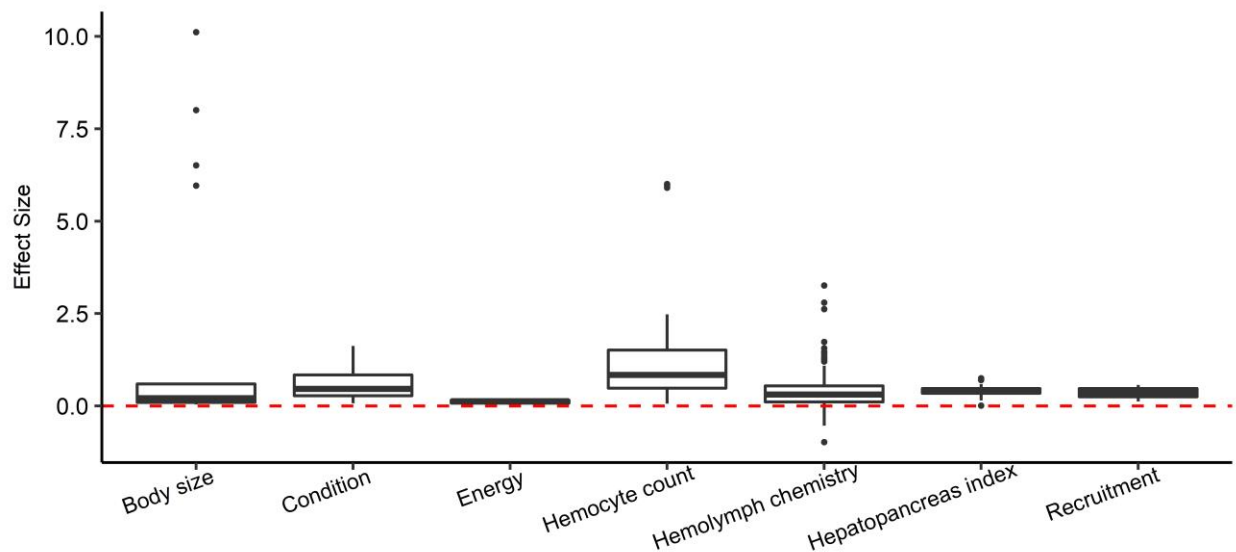


**Figure 5:** Forest plot demonstrating the effect seismic surveys have on invertebrate behavior and physiology. Effect sizes with 95% confidence intervals were plotted for each study, along with a summary random effects (RE) model with 95% confidence intervals. Weight of each study in the model is represented by dot size. Vertical dashed line indicates null effect size.



**Figure 6:** Boxplots of effect sizes observed by two invertebrate taxa in response to seismic surveys. Horizontal dashed line indicates null effect size.

A variety of behavioral and physiological parameters were evaluated for cumulative impacts from seismic surveys (Figure 7). Physiological parameters such as condition and hemocyte count had significant positive effect sizes. There were no behavioral parameters assessed in response to seismic surveys.



**Figure 7:** Boxplots of the effect sizes observed in marine invertebrates broken down by various physiological responses to seismic surveys. Horizontal dashed line indicates null effect size.

## D. DISCUSSION

Marine invertebrates are a crucial component of healthy ecosystems. For example, benthic invertebrates are responsible for maintaining oxygen concentrations in the sediment (Solan et al., 2004), while abundance and richness of marine invertebrates also increases structural complexity in coastal areas (Heck Jr and Wetstone, 1977). Despite the importance of invertebrates to marine



ecosystems, the impacts of human generated activities on their behavior and physiology is just beginning to be understood. Cumulative impacts on different taxa are challenging to study, however, meta-analysis techniques provide an opportunity to quantitatively evaluate collective effects. Here, we analyzed the effects of two anthropogenic sounds (shipping noise and seismic surveys) on the behavior and physiology of marine invertebrates.

We found that exposure to shipping noise significantly altered the foraging and antipredator behaviors of marine invertebrates. These changes in behavior were observed across taxa, with Cephalopoda and Malacostraca both displaying reduced foraging and responses to predators. Shore crabs had disrupted feeding and were slow to find shelter during exposure to shipping noise (Wale et al., 2013). Cuttlefish (*Sepia officinalis*) also display strong behavioral changes when exposed to shipping noise, changing their coloration and raising their first pair of arms more frequently during playback (Kunc et al., 2014). These reductions or changes in behaviors critical to survival could have severe fitness consequences (Anderson et al., 2011; Lagardère, 1982; Simpson et al., 2016).

Physiological responses (e.g. respiration rate, heat shock proteins, and phenoloxidase) to shipping noise by marine invertebrates are all indications of increased stress. Increased respiration rate is a typical stress response in many organisms (reviewed in Grossman, 1983), and molecular measures of stress responses have also been identified for invertebrates (Rodriguez and Le Moullac, 2000; Verghese et al., 2012). Heat shock protein 70 (Hsp 70) is an indication of stress during long-term (3 hours) heat exposure (Liberge and Barthélémy, 2007), and heat shock proteins (e.g. Hsp70, Hsp27) have been documented to be upregulated during noise exposure in the European spiny lobster (Celi et al., 2014; Filiciotto et al., 2014). In addition, phenoloxidase has been linked to organism condition factors, such as host defense and ion concentrations (Sung et al., 1998), and a stress response during high temperatures (Cheng et al., 2005) and elevated noise environments (Celi et al., 2015).

Research on the impacts of seismic surveys on marine invertebrates is more limited compared to shipping noise, as our systematic search revealed only four studies. While there were no behavioral parameters examined, physiological responses to seismic surveys provided some interesting results. Namely, hemocyte count and organism condition both had positive effect sizes. Total hemocyte count (THC) is generally used to assess stress and overall health of invertebrates (Jussila et al., 1997; Paterson et al., 2005), however our meta-analysis shows an increase in THC when exposed to seismic surveys. Additional studies are required to accurately synthesize and assess the effects of seismic surveys on the physiology of marine invertebrates.

Many marine ecosystems are under threat from rising ambient noise levels, specifically from anthropogenic sound sources including shipping and seismic surveys (Nieukirk et al., 2004; Wenz, 1972). Understanding the potentially harmful effects that increased anthropogenic noise could have on different taxa that make up marine ecosystems is critical. Past research has focused on impacts to higher trophic level organisms, predominantly marine mammals (Lusseau et al., 2009; Parks et al., 2007) and fish (Ferrari et al., 2018; Simpson et al., 2015), but research on impacts to marine invertebrates by human-generated activities is continually growing. Our analysis represents a necessary and much needed evaluation of the impacts anthropogenic activities are having on the behavior and physiology of marine invertebrates and will increase our knowledge of the ecosystem wide effects rising noise levels are having globally.

## ACKNOWLEDGMENTS

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## APPENDIX A

Table 1. The number of data points corresponding to each study used in this meta-analysis, separated by sound source.

Sound category	Authors	Number of data points
Seismic surveys	Day et al. 2016	15
	Day et al. 2017	38
	Fitzgibbon et al. 2017	222
	Przeslawski et al. 2018	18
Shipping noise	Celi et al. 2015	4
	Charifi et al. 2018	6
	Filiciotto et al. 2015	16
	Filiciotto et al. 2016	6
	Jolivet et al. 2016	5
	Kunc et al. 2014	3
	Lecchini et al. 2018	30
	Nedelec et al. 2014	2
	Tidau and Briffa 2019	2
	Wale et al. 2013a	8
	Wale et al. 2013b	3

Table 2. The specific response variables included in each broader response category, plotted in Figures 4 and 7.

Response category	Specific response variables
Anti-predator	Time to hide, time to right self, frequency of colour changes, time swimming, frequency of raised arms, total decision time to accept or reject the optimal shell
Body size	Larval size, length, width, dry mass, shell height
Condition	Adductor muscle diameter, ovary area, testes area, gonad area, gonad stage
Development	Percent of eggs that failed to develop, percent of unhatched eggs
Energy	Energy content, oxygen to nitrogen (O:N) ratio, ATP content
Foraging	Time taken to find food source, proportion of clams, number of crabs eating, number of shrimps eating, monthly mean food consumption
Heat shock proteins	Expression of heat shock protein 27, 60, 70 and 90, integrated density value
Heavy metals	Cadmium concentration in the gills or digestive gland
Hemocyte count	Total hemocytes, percent hyalinocytes, percent semigranulocytes, percent granulocytes
Hemolymph chemistry	Glucose concentration, various hemolymph components (e.g. Cl, K, Na), hemolymph pH
Hepatopancreas index	Hepatopancreas index
Metamorphosis	Percent metamorphosis success
Phenoloxidase	Phenoloxidase activity, densitometric analysis of phenoloxidase protein
Protein count	Total protein count, hemolymph protein count, brain protein count, protein concentration
Recruitment	Recruitment count, proportion of larvae settled, number of hatched larvae
Respiration rate	Oxygen uptake, respiration rate