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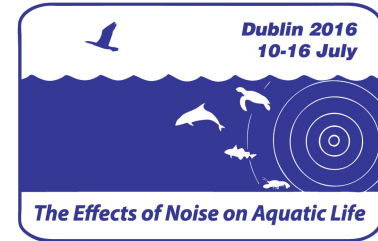
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Assessing the effect of aquatic noise on fish behavior and physiology: a meta-analysis approach

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Due to the extreme distance that sounds can travel through water, many marine species rely on the soundscape for auditory information regarding predator or prey locations, communication, and habitat selection. These species not only take advantage of the prevailing sounds but also contribute to the soundscape through their own vocalizations. Certain sounds have been shown to have negative effects on marine species, resulting in disrupted communication and unbalanced predator-prey interactions. Unfortunately, the vast majority of soundscape studies are biased towards marine mammals, and only recently has attention been directed towards the potential repercussions for fishes. In an attempt to determine the implications that changes to the soundscape may have on the fishes, a meta-analysis was conducted focusing primarily on the role that anthropogenic noises may play in altering fish behavior and physiology. The review identified 3,174 potentially relevant papers of which were 27 used. The analysis indicates that anthropogenic noise has an adverse effect on marine and freshwater fish behavior and physiology. These findings suggest that although certain species may be more susceptible to anthropogenic noise than others, the vast majority of fish have the potential to be negatively affected by noise pollution.



1. INTRODUCTION

The range and intensity of anthropogenic aquatic noise has increased considerably in recent decades, yet still little is known about its effects on fish and invertebrates (Popper and Hastings, 2009; Popper and Fay, 2011; Simmonds et al., 2014). This noise contains no intentional information and is thought of as noise pollution, commonly attributed to increased shipping and industrial activity (Bass and Clark, 2003; Popper and Hastings, 2009; Pijanowski et al., 2011). Unfortunately for marine species, the adaptations that have increased their ability to detect and produce sound have also made them more susceptible to environmental stressors such as noise (Popper and Hastings, 2009; Picciulin et al., 2010). Depending on the intensity and duration of exposure, noise pollution has the potential to temporarily, or even permanently, alter auditory thresholds, as well as mask the detection of important environmental cues (Bass and Clark, 2003; Ladich, 2008; Popper and Hastings, 2009; Picciulin et al., 2010). Despite the concerns regarding increasing anthropogenic noise, the monitoring and regulation of this pollutant has been limited (Simmonds et al., 2014). This is in part due to a lack of knowledge and understanding of the ecological effects of noise on marine organisms (Bass and Clark, 2003; Popper and Hastings, 2009). However, recent reviews suggest that noise disturbance would likely first lead to physiological and behavioral changes (Kight and Swaddle, 2011; Gedamke et al., 2016).

In an attempt to determine the implications that changes to the soundscape may have on fishes, we carried out a meta-analysis, which is an effective method for assessing ecological trends (Mann, 1990; Gurevitch et al., 1992; Vander Werf, 1992; Fernandez-Duque and Valeggia, 1994; Poulin, 1994). We assessed 3,174 peer-reviewed studies focusing on the impact of aquatic noise on fish behavior or physiology. Twenty-seven of these studies met our search criteria and were used to develop models addressing the effect of anthropogenic noise on fish behavior, and the effect of anthropogenic noise on fish physiology.

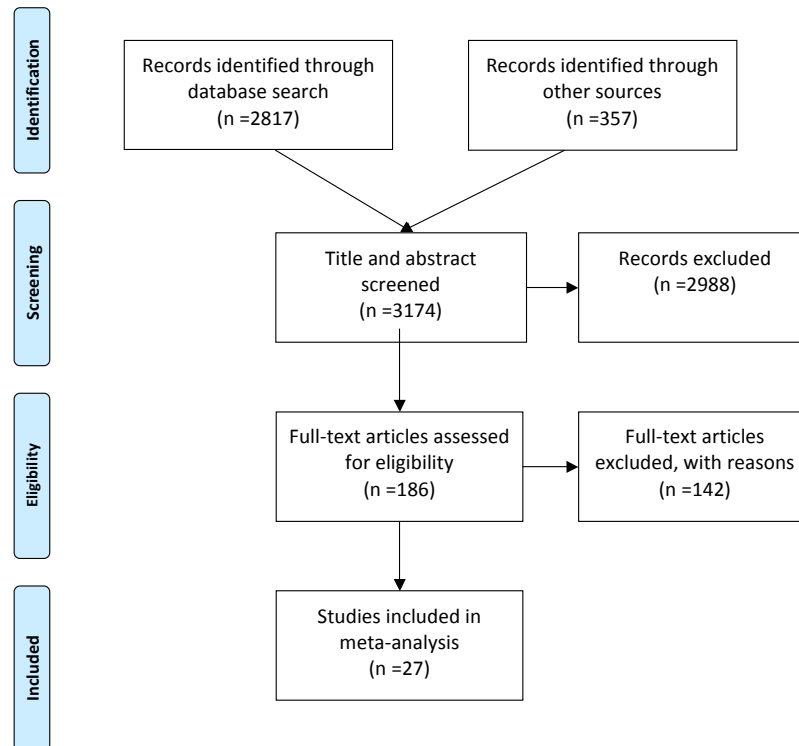
2. METHODS

A. SYSTEMATIC LITERATURE SEARCH

A systematic literature search was conducted using Thompson's ISI Web of Science within the following subject areas 'acoustics', 'zoology', 'ecology', 'environmental sciences', 'ichthyology', 'biodiversity conservation', 'evolutionary biology', 'marine biology', and 'freshwater biology'. Search results were limited to peer-reviewed articles published between 1950 and 2015. The specific search terms were 'fish' and 'noise or sound or acoustic or ecoacoustics or bioacoustics' and 'behav* or physiol* or response or morphology', which returned 2,817 potentially relevant peer-reviewed articles (Fig. 1). Other search engines identified an additional 357 potentially relevant peer-reviewed articles. The titles and abstracts of the 3,174 studies were reviewed to determine which papers addressed the effects of anthropogenic noise on fish behavior or physiology. This process identified 186 relevant papers which were evaluated to further identify those that met our search criteria of: original research, focused on the impact on behavior or physiology, included the source of sound, used a control, included the mean value, the standard error, the standard deviation, and the sample size. The 27 studies that met these requirements were then systematically characterized according to 15 attributes, including, parameters measured, genus studied, fish origin, stimulus type and duration, testing facility, location, water quality and fish condition. We then extracted the sample size, mean, and standard deviation of the treatment and control groups from each of the 27 studies. All

data were obtained from tables and text when possible, and if necessary GraphClick, a reliable and accurate extraction software, was used (Arizona-Software, 2008; Boyle et al., 2013).

To account for the large amount of variation present within the response variables, the directionality of each study was determined to ensure that negative effect sizes represented negative responses, and positive effect sizes represented positive responses. For example, with a response variable like growth rate, an increase would result in a positive effect size, while an increase in a response variable like cortisol, a common stress hormone, would lead to a positive effect size, despite being a negative response. Accounting for the directionality of each response variable is thus a critical step for a meta-analysis of this nature. Additionally, in rare cases when studies evaluated the effect of anthropogenic noise on both behavior and physiology, we treated each evaluation independently as the overall effect sizes of behavior and physiology models were not pooled during our analysis.



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

Figure 1. A PRISMA diagram outlining the selection processes of papers for the meta-analysis.

B. EFFECT SIZE CALCULATION

We used the Metafor package in R to calculate the effect sizes and variances for each study (Viechtbauer, 2010; R Core Team, 2015). Mean difference (md) was calculated using Eq. (1), where \bar{Y}_1 and \bar{Y}_2 are the mean values of the treatment and control group.

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

The standardized mean difference (Hedge's d), which is an indication of the overall effect and weights of studies based on their sample sizes and standard deviations, was determined using Eq. (2). Again, \bar{Y}_1 and \bar{Y}_2 are the treatment and control means, while sample sizes are indicated by n_1 and n_2 with standard deviations s_1 and s_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 Hedge's d was determined via Eq. (3) (Hedges and Olkin, 1985)

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

C. STATISTICAL ANALYSIS

The analysis was conducted in R-studio (R Core Team, 2015; R Studio Team, 2015). Forest and funnel plots were generated using the 'metafor' package, while boxplots were made using the 'ggplot2' package (Wickman, 2009; Viechtbauer, 2010).

The effect of anthropogenic noise on fish behavior, and the effect of anthropogenic noise on fish physiology were determined using separate but identical analyses on each of the appropriate data sets. Shapiro–Wilk tests were used to determine that the effect sizes were non-normal, despite the use of standard transformations (data not shown); as such non-parametric tests were utilized when appropriate. A Kruskal-Wallis rank sum test was used to determine if there were significant differences between the effect sizes of wild caught and lab-raised fishes, and between marine and freshwater fishes (Signorell, 2009). Forest plots determined the summary effect and confidence intervals of each model, and funnel plots established if any potential publication bias was present. Restricted maximum likelihood (REML) random-effect

models, known as omnibus tests, were run to test the significance of the overall models using the *mareg* function in the 'MAAd' package (Del Re and Hoyt, 2014). Model heterogeneity, an indication of the inconsistencies of effect sizes across studies, was determined using the *confint* function within the 'metafor' package (Viechtbauer, 2010).

3. RESULTS

Twenty-seven of the 3,174 potentially relevant studies met our search criteria; of which 9 focused on the effects of noise on fish behavior and 18 addressed the effects of noise on fish physiology.

Funnel plots indicated that there was no publication bias within studies focusing on fish physiology, and a minor but acceptable amount of publication bias present within studies focusing on fish behavior (data not shown).

Of the 15 attributes identified, wild or lab-raised, and freshwater or marine, were the two attributes most likely to impact the overall effect size. However, neither of these two criteria had an effect on the observed effect sizes (Fig. 2).

Forest plots indicated that anthropogenic noise had a significantly negative effect on fish behavior (Fig. 3). All individual study effects sizes were negative, and 4 had confidence intervals that did not overlap zero (Fig. 3). The overall effect size of -3.04 with 95% confidence intervals (CI) of -5.42 and -0.65 indicates that there is a negative impact of -3.04 standard deviations on behavior when fish are exposed to anthropogenic noise, compared to control groups.

Fish physiology was also negatively affected by anthropogenic noise (Fig. 4). All studies had negative effect sizes, and 7 had confidence intervals that did not overlap zero (Fig. 4). The overall effect size of -1.02 (95% CI of -1.43 and -0.61) indicated that when fishes are exposed to anthropogenic noise there is a negative response of -1.02 standard deviations in fish physiology relative to controls.

The omnibus tests, run on the effect sizes and associated variances, indicated that the negative responses observed in the forest plots were significant whether fish behavior or physiology was considered, as shown in Table 1.

Study heterogeneity was $I^2=74.12\%$ (CI 54%, 94%) and $I^2=98.80\%$ (CI 97%, 100%) for omnibus models based on fish physiological and behavioral responses, respectively. The study heterogeneities are provided in Table 1.

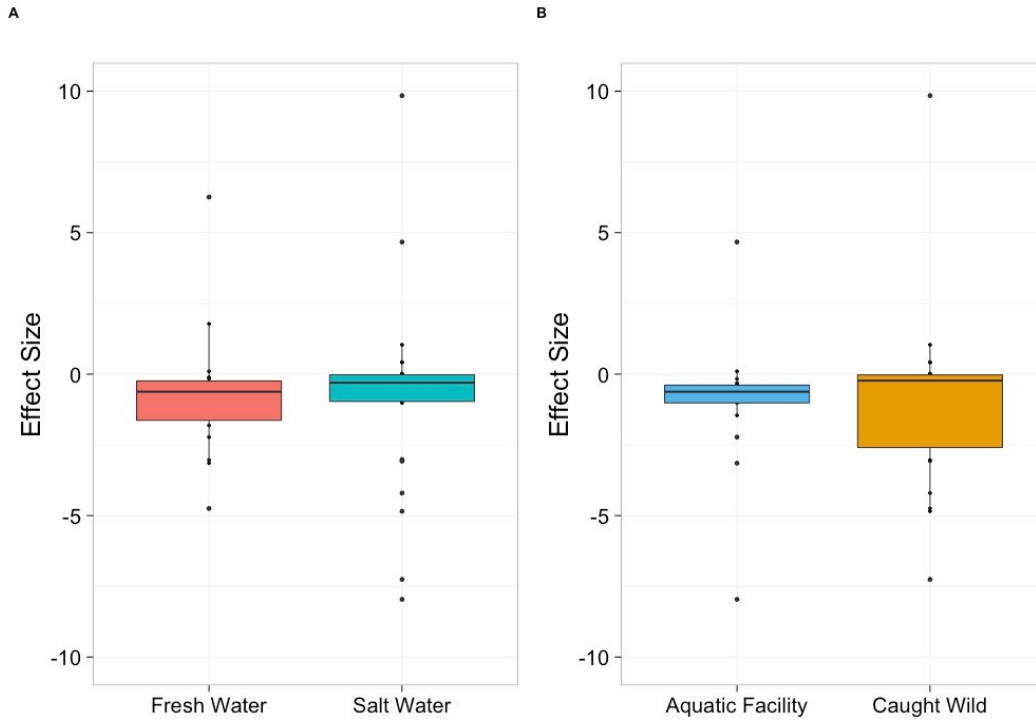


Figure 2 A) Boxplots of the effect sizes observed in marine and freshwater fishes. Kruskal-Wallis chi-squared = 0.26, *df* = 1, *p* > 0.50. B) Boxplots of the effect sizes observed in wild fish and fish acquired from aquatic facilities. Kruskal-Wallis chi-squared = 1.05, *df* = 1, *p* > 0.30.

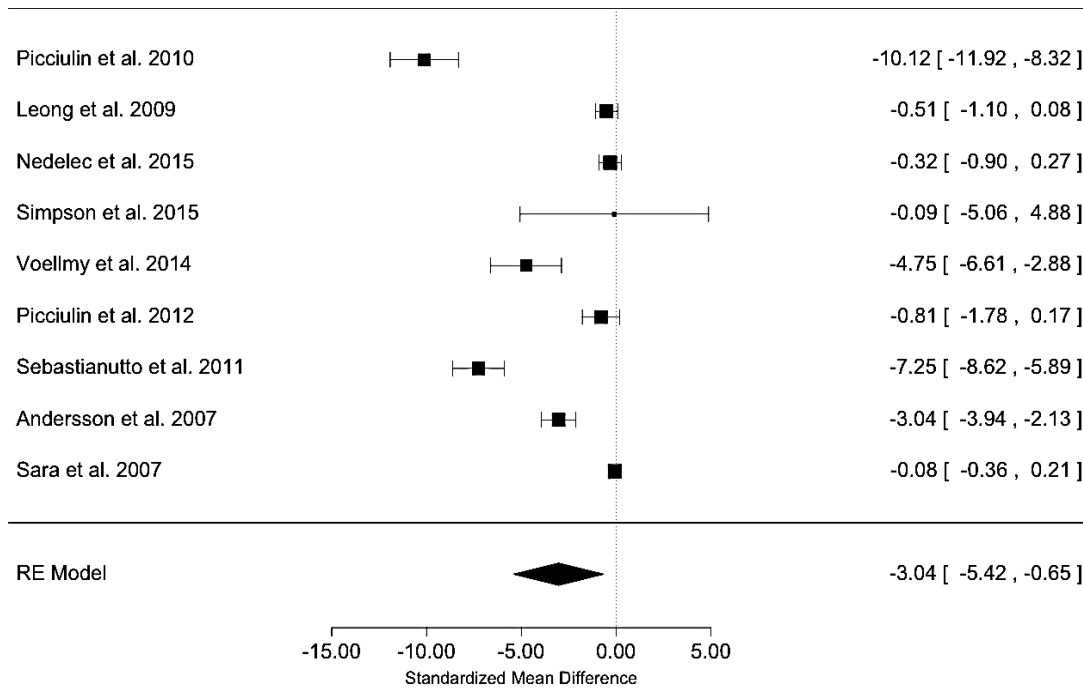


Figure 3. Forest plot of effect sizes (standardized mean differences) and summary random effects (RE) model with 95% confidence intervals for studies that report data on the effects of anthropogenic noise on fish behavior.

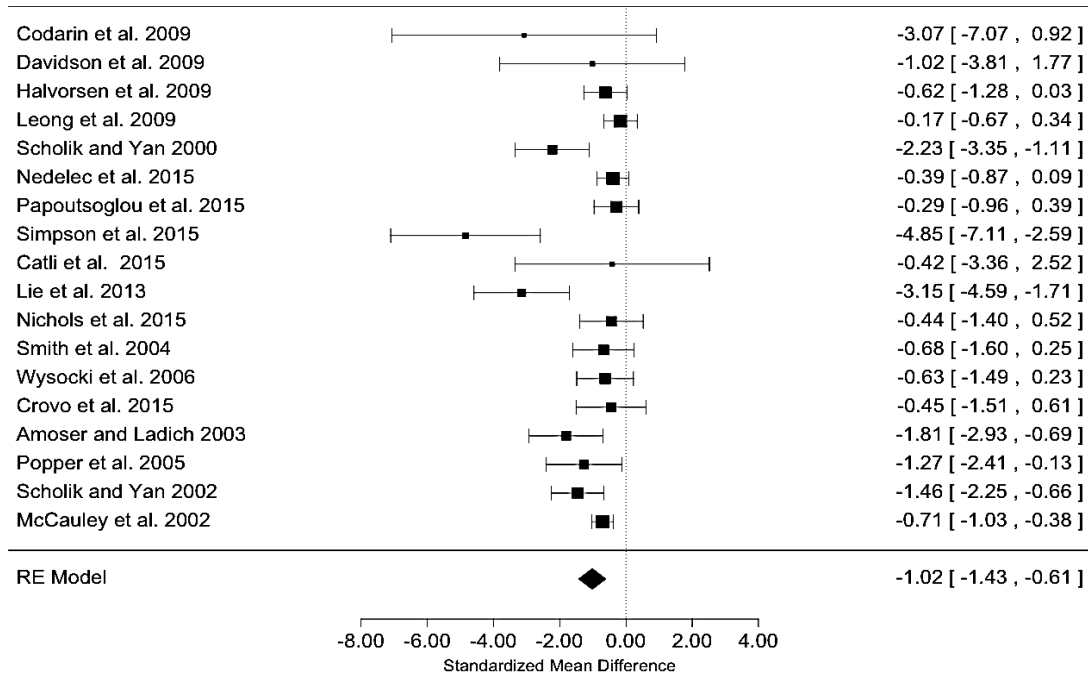


Figure 4. Forest plot of effect sizes (standardized mean differences) and summary random effects (RE) model with 95% confidence intervals for studies that report data on the effects of anthropogenic noise on fish physiology.

Table 1. Omnibus test and model heterogeneity, performed on both physiological and behavioral models. C.I = Confidence interval.

Model	Omnibus Estimate	Lower C.I	Upper C.I	p-value	Model Heterogeneity	Heterogeneity Lower C.I	Heterogeneity Upper C.I
Physiology	-1.02	-1.43	-0.61	0.00	74.12	54.43	94.06
Behavior	-3.04	-5.43	-0.65	0.01	98.80	97.21	99.68

4. CONCLUSION

Given the growing concern of how aquatic species will be affected by increasing anthropogenic noise, researchers are continually looking for innovative ways to evaluate this issue (Popper and Hastings, 2009; Popper and Fay, 2011). Meta-analyses are an ideal way to combine results from many studies in a robust quantitative fashion. We conducted a meta-analysis to address the impact of anthropogenic noise on fish behavior and physiology. This analysis summarized the overall effect of anthropogenic noise on multiple marine and freshwater fishes under various experimental conditions.

Forest plots and associated models showed that anthropogenic noise has an adverse effect on fish behavior and physiology (Fig. 3; Fig. 4). A negative overall effect size was observed for both behavioral and physiological models, indicating that the potential for anthropogenic noise to negatively impact fishes is likely not limited to specific responses. These findings suggest that

negative effects could occur regardless of the species studied, their source habitats, or the experimental conditions considered. Although the vast majority of the observed responses are associated with an inability to function properly, it is important to note that not all of these negative responses will result in permanent damage (Bass and Clark, 2003; Ladich, 2008; Popper and Hastings, 2009; Picciulin et al., 2010).

As heterogeneity (I^2) values of 75% and above are considered large, both models had high heterogeneity, which is indicative of substantial amounts of between sample variation (Del Re, 2015). Strictly speaking, low I^2 values indicate that the vast majority of the heterogeneity is due to sampling error, while high I^2 values suggest that heterogeneity is due to differences between studies (Del Re, 2015). Although a potential concern, high heterogeneity values are understandable given the diversity of response variables measured and the relatively low number of studies considered (Ioannidis et al., 2007). As behavioral responses included gross motor movements, nesting activities, agonistic encounters, and predator-prey interactions, a large amount of uncertainty is not surprising. A similar but lesser trend was observed within the physiological model, as an I^2 value of 74% was observed, compared to 99% in the case of the behavioral model, as seen in Table 1. Again, given the large number of response variables, the variability in testing conditions, and the relatively small sample size, a considerable amount of heterogeneity is not unexpected (Ioannidis et al., 2007).

The similarity between the effect sizes of marine and freshwater species is not surprising, as they share many similar characteristics, as well as a common ancestry (Vega and Wiens, 2012). This lack of variation indicates that species in both marine and fresh water systems will respond similarly to anthropogenic noises.

These results support Popper and Hastings' (2009) increasing concern about the potentially negative effects of anthropogenic noise on fishes. Fishes, across a variety of genera, are likely negatively affected by continuously increasing noise pollution. It is probable that the once advantageous adaptations that have allowed so many species to detect sound are now causing them to be vulnerable to noise pollution and in turn, less aware of environmental cues (Ladich, 2008; Popper and Hastings, 2009).

A major limitation when considering how to best monitor, regulate and potentially mitigate the increasing levels of anthropogenic noise globally has been the lack of understanding of how sound affects marine organisms (Bass and Clark, 2003; Popper and Hastings, 2009; Simmonds et al., 2014). This meta-analysis suggests that anthropogenic noise will negatively affect a broad range of fish genera, and decrease an individual's abilities to interact with conspecifics, forage efficiently, produce viable offspring, and maintain healthy growth. These impacts will likely influence both wild and lab-raised fish, and occur regardless of the body of water considered. As noise pollution is increasing globally, this study should serve as a warning, and echo the growing concerns regarding the potentially dire consequences for the marine ecosystem.

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