Great Lakes Institute for Environmental
Research Publications

Great Lakes Institute for Environmental
Research

1-1-2019

Behavioural and morphological changes in fish exposed to ecologically relevant boat noises

Megan F. Mickle
University of Windsor

Christopher M. Harris
University of Windsor

Oliver P. Love
University of Windsor

Dennis M. Higgs
University of Windsor

Follow this and additional works at: https://scholar.uwindsor.ca/glierpub

Part of the Biochemistry, Biophysics, and Structural Biology Commons, Biodiversity Commons, Biology Commons, and the Marine Biology Commons

Canadian Journal of Fisheries and Aquatic Sciences

Behavioural and morphological changes in fish exposed to ecologically-relevant boat noises

Journal:	*Canadian Journal of Fisheries and Aquatic Sciences*
Manuscript ID	cjfas-2018-0258.R1
Manuscript Type:	Article
Date Submitted by the Author:	25-Nov-2018
Complete List of Authors:	Mickle, Megan; University of Windsor, Biology Harris, Christopher; University of Windsor Love, Oliver; Univ Windsor, Higgs, Dennis; University of Windsor, Biology
Keyword:	BEHAVIOR < General, PHYSIOLOGY < General, FRESHWATER FISHES < General, Anthropogenic noise, MORPHOLOGY < General
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

SCHOLARONE™
Manuscripts

1

2 **Behavioural and morphological changes in fish exposed to ecologically-relevant boat noises**

3

4
5
6
7

8 Megan F. Mickle[1], Christopher M. Harris[1,2], Oliver P. Love[1,2], Dennis M. Higgs[1]

9
10

11 [1]Department of Biological Sciences, University of Windsor, 401 Sunset Avenue, Windsor,
12 Ontario N9B 3P4, Canada

13 [2]Great Lakes Institute for Environmental Research (GLIER), University of Windsor, 401 Sunset
14 Avenue, Windsor, Ontario N9B 3P4, Canada

15 Christopher Harris: harris2c@uwindsor.ca

16 Oliver Love: olove@uwindsor.ca

17 Dennis Higgs: dhiggs@uwindsor.ca

18
19 Corresponding author: Megan Mickle: micklem@uwindsor.ca, 519-253-3000 x 4039

20

21 Keywords:

22   - Behaviour

23   - Physiology

24   - Glucocorticoids

25   - Freshwater

26   - Bioaccoustics

27   - Anthropogenic noise

28   - Ciliary bundles

29

30

31

32

33   **Abstract**

34   There is increasing concern about the effect of underwater noise on fish due to rising

35   levels of anthropogenic noise. We performed experiments on the black bullhead *(Ameiurus*

36   *melas)*, a species with known hearing specializations and located within the Laurentian Great

37   Lakes where there is significant commercial and recreational boat traffic. We tested and

38   compared physiology (baseline cortisol), behaviour (activity, sheltering) and morphology (cilary

39   bundles of hair cells) of bullhead to boat noise. At 140 dB re 1 μPa (-54.84 dB re 1m s^{-2}) we saw

40   clear behavioural effects in terms of both activity and sheltering levels despite no obvious

41   morphological or physiological stress. Following both short and long period acute exposure to

42   higher — but environmentally relevant — noise levels, bullhead were less active and sheltered

43   more and also exhibited a decrease in cilary bundles. These results suggest that there are

44   sublethal effects of anthropogenic noise on fish behaviour and cilary bundles which may have

45   direct implications on population health. Moreover, commonly used metrics such as stress

46   hormones may not always offer the most relevant biomarker of the response to anthropogenic

47   boat noise.

Introduction:

Anthropogenic noise is now common in aquatic ecosystems, although the effects this has on aquatic animals, particularly freshwater fishes, remains unclear (Slabbekoorn et al. 2010; Popper & Hawkins 2012). There has been a notable increase in anthropogenic noise due to industrialization primarily caused by the expansion of transport networks and various resource extraction methods (Wale et al. 2013; Solan et al. 2016), all of which have the potential to disrupt acoustic communications (Wysocki et al. 2006; Popper & Hastings 2009; Wale et al. 2013). Although there has been a greater focus on effects of noise sources such as sonar, airguns and pile driving (Shannon et al. 2015), recreational and commercial boats are the predominant source of anthropogenic noise at low frequencies underwater (Ross 1976; Dyndo et al. 2015; Nichols et al. 2015; Shannon et al. 2015; Solan et al. 2016), having considerable overlap with the sound production and hearing range of most fish species examined to date (20-1000 Hz) (Kasumyan 2005; Ladich & Fay 2013; Nichols et al. 2015).

Noise pollution research is well studied in marine environments with a particular focus on marine mammals and fish (Popper 2003; Slabbekoorn et al. 2010), indicating that noise impacts on animals can range from non-lethal behavioural and physiological stressors to death (Weilgart 2007; Mickle & Higgs 2017). Research that has been performed regarding noise impacts on freshwater fish is generally focused on aquaria fish such as: goldfish (*Carassius auratus;* Wysocki & Ladich 2005; Smith 2003), zebrafish (*Danio rerio*; Neo et al. 2015; Sabet et al. 2015) and cichlids (Cichlidae; Hastings et al. 1996; Bruintjes & Radford 2013), resulting in a gap in our knowledge of noise effects on wild, economically important freshwater fish. Freshwater ecosystems have a disproportionately high fish diversity (Combes 2003) but comparatively less attention on effects of anthropogenic noise. Given the importance of both

71   freshwater lakes and fish to shipping and the economy, more research is also needed to

72   determine the full impact of shipping noise in these high traffic environments.

73   Hearing is an important sensory modality in fish for communication and orientation with

74   a great diversity in hearing abilities between species (Hawkins 1981; Aalbers & Drawbridge

75   2008; Fay 2009). To date the majority of research regarding noise pollution incorporates either

76   behavioural or physiological measures alone, but seldom have single studies integrated

77   techniques (Cooke et al. 2014; Mickle & Higgs 2017). Previous work on fishes leads to the

78   suggestion that some individuals exposed to anthropogenic noise show physiological stress

79   responses such as increased levels of stress hormones (Barcellos et al. 2007), change in cardiac

80   output (Graham & Cooke 2008), and further hypothesize changes in gene expression and

81   immune function (Mommsen et al. 1999; Barton et al. 2002; Shannon et al. 2015). Fish exposed

82   to loud sounds may also suffer from physical impairments such as hair cell damage (Hastings et

83   al. 1996; Wysocki et al. 2007) or a shift in their hearing threshold (Enger 1981; Smith et al.

84   2006). Finally, behavioural changes have also been seen in response to loud sounds with changes

85   in overall behaviour level (Ona & Godø 1990), feeding behaviour (Payne et al. 2014) and

86   predator/prey interactions (Sabet et al. 2015; Simpson 2016). While these individual effects are

87   informative, more integrative work may more accurately identify possible noise effects on fish.

88   Thus, increased efforts on integrative studies in freshwater fish will help to better understand

89   possible effects on anthropogenic noise in aquatic environments.

90   Here we take an integrative approach to examine potential impacts of noise on the

91   phenotypic responses of black bullhead *(Ameiurus melas)*, a species hypothesized to have

92   specialized hearing capabilities based on previous research on Siluriformes (Poggendorf 1952;

93   Kleerkoper & Roggenkamp 1959; Lechenr & Ladich 2008) and located within the Laurentian

94 Great Lakes where there is significant commercial and recreational boat traffic. To obtain a

95 holistic measure of the phenotypic response to noise in this species, we measured behavioural,

96 physiological and morphological changes across biologically relevant noise levels. First, we

97 examined the impacts of noise levels at 140 dB re 1μPa RMS (ranging in frequency from 100-

98 10,000 Hz) on bullhead behaviour and physiology, and then to further explore these results we

99 exposed fish to differing intensities of noise (160 and 170 dB re 1μPa RMS; 100-10,000 Hz)

100 across two time points, 1 hour and 24 hours. We hypothesized that bullhead exposed to 140 dB

101 re 1μPa would exhibit behavioural changes, while bullhead exposed to 160 and 170 dB re 1μPa

102 would exhibit changes in behaviour, physiology and morphology in response to noise. More

103 specifically, we hypothesized that bullhead under acute noise exposure would exhibit

104 behavioural and physiological responses to noise, while those with chronic exposure will exhibit

105 physical damage to hearing-related tissues.

106 **Methods**

107 *Experimental Design:*

108 All work was conducted under approved Canadian Council for Animal Care (CCAC) protocols

109 (University of Windsor AUPP 14-11). Non-reproductive black bullhead, ranging from 22.02-

110 117.60g were obtained from a fish farm in Harrow, Essex County, Ontario (42°01'14.5"N

111 83°00'04.1"W). Due to the constraints of catching wild/farmed fish these species were a range in

112 size. Fish were housed at a temperature of 22.2°C and a pH of 6.5-7 in animal quarters at the

113 University of Windsor. The fish were fed daily and kept at a 12:12 light-dark cycle to mimic

114 natural conditions, however as these species prefer dark environments all housing tanks were

115 covered with black garbage bags and opaque tank covers.

116   Noise levels were collected from housing and experimental tanks using a hydrophone

117   system (Inter Ocean system inc. – Acoustic Calibration and System Model 902) and, where

118   possible, using a waterproofed accelerometer (model 4524 cubic triaxial deltatron, Brüel &

119   Kjær) to estimate noise levels as pressure and particle motion. Both the hydrophone and the

120   accelerometer were suspended in the middle of the tank and both were used because the fish ear

121   is fundamentally responsive to acceleration but pressure measurements are more easily

122   understood for those concerned about noise exposure in a field setting (see Hawkins & Popper

123   2018). We also recognize the inherent problems with complex acoustics in small tanks

124   (Parvulescu 1964; Rogers et al. 2015) but the current set of experiments would not be possible in

125   a field setting and can still provide useful information about possible noise effects. The

126   background noise in the experimental tanks was below 120 dB re 1 μPa RMS and below the

127   noise floor of the accelerometer system. Bullhead likely detect both pressure and particle

128   components of sound, as can other Ostariophysan fishes (Higgs et al. 2006), but because we

129   ultimately want to refer these results to levels experienced in the natural habitats where

130   anthropogenic sound is most relevant, and where particle motion is still difficult to accurately

131   measure, more emphasis is placed on the pressure units.

132   Two noise experiments were conducted, the first consisted of 140 dB re 1 μPa treatment

133   and the second consisted of a 160/170 dB re 1 μPa noise treatment, however both treatments

134   played the same boat noise file (Fig. 1). Boat noise was recorded from a recreational vessel using

135   a hydrophone (Loggerhead Instruments, Model # HTI-96-Min/3V/Exp/LED) placed at a depth of

136   approximately 2m at a distance of 4m from a boat launch in a local Great Lakes habitat (Chewitt

137   bay, Ontario, Canada). This sound file was then played through an underwater speaker (UW-30,

138   Lubell Labs) at 140 (equivalent to -54.84 dB re 1m s^{-2}), 160 (equivalent to -50.61 dB re 1m s^{-2})

139   and 170 dB re 1µ Pa (equivalent to -46.55 dB re 1m s⁻²) (all sound levels are in RMS; Fig. 1).

140   Only one recording was chosen to standardize the exposure across treatments so we do not

141   expect these results to necessarily reflect all boat recordings possible (see Slabbekoorn & Bouton

142   2008).

143   The experimental setup included an underwater speaker (Electro-Voice UW-30),

144   connected to an amplifier (Scosche SA300), a 12 Volt PBS car battery for power and an mp3

145   player to play the noise (Sony Walkman NWZ-E464). The speaker was placed in the middle of

146   the tank and background noise was quantified using a hydrophone which measured decibel levels

147   at 8 locations and two depths in the tank, and reliably ranged from 116- 122 dB re 1µPa.

148   During the 140 dB re 1 µPa level experiment, six individual bullhead (total n=60),

149   similar in size, were randomly collected from housing tanks and added into separate plastic

150   experimental tanks (55 liters) in a dark room equipped with red light, a PVC shelter, an

151   underwater speaker and single air stone in each tank. During the high intensity noise experiment

152   (160/170 dB re 1 µPa), three fish (total n=24) similar in size were added into one plastic

153   experimental tank, with the same set-up as the lower intensity experiment, however equipped

154   with three PVC tubes so each fish could have an individual shelter (Fig. 2).

155   *Behavioural Assays:*

156   During the first experiment (140 dB re 1µ Pa level noise exposure) (n=60), six bullhead were

157   given an acclimation time of one hour after which a baseline control treatment took place for

158   four hours followed by a four-hour noise treatment (Fig. 3a). There were two separate controls

159   for this experiment, a "baseline control" and a "non-treatment control". The baseline control took

160   place after the acclimation period but before the noise was played (Fig. 3A), while the non-

161   treatment control replicated entire experimental conditions without the presence of noise (Fig. 3).

162   To quantify a change in behaviour, experiments were recorded using a GoPro Hero3+ (Go Pro).

163   Sheltering and general swimming behaviours were analyzed and compared during the last hour

164   of both the baseline control and noise treatments. We quantified a sheltering response when the

165   fish were residing in Polyvinyl chloride (PVC) tubing (one tube in each tank) and activity levels

166   were quantified as a measure of time spent swimming throughout the videos. Activity and

167   sheltering accounted for the total behaviours observed during the experiment.

168   Based on the results from 140 dB re 1µ Pa we decided to perform a second experiment to

169   observe the impacts of higher noise levels on bullhead (160 dB re 1µ Pa and 170 dB re 1µ Pa).

170   During the second experiment black bullhead (n=24) were exposed to either 160 or 170 dB re 1µ

171   Pa of boat noise for either one hour (short period acute noise exposure) or twenty-four hours

172   (long-term acute noise exposure). Three bullhead were placed in the experiment tank (as opposed

173   to six bullhead in individual tanks as performed in the lower intensity experiment) and allowed

174   to acclimate for one hour before noise treatment (at either 160 or 170 dB re 1µ Pa) began. Two

175   separate controls (baseline and non-treatment) were also implemented for this experiment.

176   During short period noise exposure (1 hr), fish acclimated for one hour, after which a one-hour

177   baseline control treatment began followed by one hour of boat noise (at either 160 or 170 dB re

178   1µ Pa) (Fig. 3b). During the long-term noise exposure (24 hr), fish were also allowed to

179   acclimate for one hour, followed by a one-hour baseline control treatment and then 24 hours of

180   boat noise (at either 160 or 170 dB re 1µ Pa) (Fig. 3c). To keep consistency in behavioural

181   videos, we recorded the fishes' behaviour during the last hour of the long-term noise experiment.

182   There were two experimental replicates (n=6) for both short and long-term acute exposure

183   experiments, at both 160 and 170 dB re 1µPa, totalling 24 fish (Fig. 3c). We quantified

184   sheltering response and activity levels using the same methods presented in experiment 1. Noise

185   experiments started at approximately the same time each day to avoid diurnal differences in

186   behaviour

187   *Physiological Assays:*

188   At the end of each experiment, bullhead were anaesthetized using 2-phenoxy ethanol (SIGMA-

189   ALDRICH Product #: 1 mL of 2-phenoxy ethanol per 2 L of water) and each tail was removed

190   within two minutes so that blood from the caudal artery could be collected using a heparanized

191   capillary vial. Once blood was collected, the fish were decapitated and heads fully submerged in

192   paraformaldehyde (4%) for preservation before further dissection of ears. Plasma was isolated

193   via centrifugation and cortisol was subsequently extracted from the plasma using a standard

194   ELISA protocol. Cortisol levels were determined using a commercially-available enzyme

195   immunoassay (Cayman Chemical Company, Ann Arbor, MI) with assays performed according

196   to kit instructions. Before beginning assays, a pool of black bullhead plasma was assayed both

197   raw and after dichloromethane extraction. Serial dilutions of both were found to be parallel to the

198   standard curve. As extracted samples showed reduced values due to recovery losses and raw

199   plasma showed no indication of interference, samples were run on raw plasma without

200   extraction. To ensure sample cortisol values fell within the kit detection range, bullhead samples

201   were assayed at 1:20 dilution (10uL of plasma and 190uL of assay buffer). Sample

202   concentrations were determined using an 8 point standard curve run in duplicate on each assay

203   plate. Standards ranged in concentration from 4000pg/mL to 6.6pg/mL while the minimum

204   detection limit of the assay is 35pg/mL. At the end of incubation, the absorbance values for each

205   well were measured at 412nm using a BioTek Synergy H1 plate reader. All samples were

206   assayed across 7 plates yielding an inter-assay variation of 19.96% and intra-assay variation of

207   5.3% for bullhead.

Ciliary Bundle Counts:

209   The catfish saccule is an irregular structure, twice the length of the lagena with rounded

210   anterior/posterior ends (Jenkins 1977), the saccule location in bullhead was determined based on

211   the schematic depicted in Jenkins (1977) (Fig. 4c). Saccules were dissected (using a Leica L2

212   10445930 dissecting scope) from 1 of every 6 bullhead ears randomly selected from the low

213   intensity noise experiment (total n=6), and 1 of every 3 bullhead from the high intensity noise

214   experiment (total n=8). After saccules were collected, they were preserved in paraformaldehyde

215   (4%) until stained with 12.5 µl of fluorescent green phalloidin mixed with 200 µl of phosphate

216   buffer (Higgs et al. 2002). Once saccules were properly stained, ciliary bundles of hair cells were

217   visualized through images collected from a Leica microscope, (Leica DM IRB inverted

218   fluorescence microscope, Las A.F. 4.5). As there are thousands of ciliary bundles of hair cells

219   present along the saccular epithelium (Higgs et al. 2003), ciliary bundles were counted in three

220   regions along the anterior, middle and posterior saccule using a magnified view of the

221   epithelium. Images were imported into Adobe Photoshop (V3.0; Adobe Systems) to create 3

222   identical boxes of 225 µm^2 cm in size (in magnified view) representing 19% of the total saccular

223   area (Higgs et al. 2003) (Fig. 4). Ciliary bundles within each box were then counted using Image

224   J software (NIH) (Fig. 4). Hair cell damage was characterized as a difference in absolute number

225   of ciliary bundles between fish exposed to noise and control fish. Comparisons in ciliary bundles

226   of hair cell number were made between bullhead in the no-treatment control and sound exposure

227   experiments.

228   *Statistical Analyses*

229   Both controls (baseline and no- noise treatment) in the two experiments yielded similar

230   behavioural results, therefore for the purposes of this study, statistics are only reported for

231   comparisons between the baseline control and noise exposure treatments. There was no

232   difference in activity levels between the acclimation period and the no-noise control period, thus

233   time of residence in the tank was accounted for. Once data were collected, a one-way analysis of

234   variance (ANOVA), designating fish ID as a random factor, was performed using SPSS (IBM,

235   2014) to analyze behavioural differences of black bullhead when exposed to noise. Ciliary

236   bundle data were analyzed using an independent sample t-test between no-noise controls and

237   noise exposures, designating hair cell position as a random factor. To examine differences in

238   cortisol levels, data were log-transformed (as cortisol data were not normally distributed) and an

239   ANOVA was performed on differences between no-noise control animals and noise exposed

240   animals. The dependent variables in this experiment were: behavioural markers

241   (activity/sheltering response), cortisol levels and ciliary bundle count. The fixed factor in the low

242   intensity treatment consisted of sound exposure (no-noise control or 140 dB re 1µPa),

243   During the second high-intensity noise experiment (160 and 170 dB re 1 µPa), a one-way

244   ANOVA was also used to examine behavioural differences of bullhead during baseline control

245   and noise treatments. We used a Tukey-post hoc test to further investigate where differences

246   were present. Cortisol data were log transformed and analyzed using an ANOVA and ciliary

247   bundle data were compared using an independent sample t-test. The dependent variables in the

248   high intensity treatment were: activity/sheltering, cortisol levels and ciliary bundle counts,

249   however the fixed factor consisted of sound exposure (control or 160 or 170 dB re 1µPa) and

250   time of exposure (short and long period acute exposure).

251 **Results:**

252 When exposed to 140 dB re 1 μPa boat noise bullhead exhibited significant changes in

253 behavioural characteristics. Activity levels decreased from 2.63 to 0.97 (+/- 0.43 SEM) minutes

254 per hour when fish were exposed to boat noise played at 140 dB re 1μPa ($F_{1,5}$=8.4, p=0.034; Fig.

255 5a). Sheltering behaviour increased from 21.37 to 24.27 (+/- 1.21913 SEM) minutes per hour

256 when fish were exposed to noise ($F_{1,5}$=8.6, p=0.033; Fig. 5b). There was no significant difference

257 in cortisol levels relative to resting levels ($F_{8,50}$=4.2, p=0.184; Fig. 5c) exposed to 140 dB re

258 1μPa for four hours. When comparing ciliary bundle data in the control and noise treatment

259 during the 140 dB re 1μ Pa noise exposure there was no significant difference in counts (t_6=

260 0.78, p=0.902; Fig. 5d).

261   During the 160 and 170 dB re 1μ Pa noise exposure treatments, differences were present

262 within the short period acute exposure experiment (1 hour) for both activity and sheltering.

263 Activity levels significantly decreased from 21.69 to 1.97 (+/-1.0 SEM) minutes per hour during

264 160 dB re 1μ Pa exposure and from 21.69 to 8.90 (+/-1.0 SEM) minutes at 170 dB re 1μPa ($F_{2,12}$

265 = 32.987, p<0.001; Fig. 6a). Sheltering behaviour significantly increased from 27.13 to 57.70

266 (+/- 0.80509 SEM) minutes during 160 dB re 1μ Pa and from 27.13 to 50.70 (+/- 1.85 SEM)

267 minutes per hour during 170 dB re 1μ Pa when bullhead were exposed to noise ($F_{2,12}$ = 11.236,

268 p<0.001; Fig. 6b). During the long period acute exposure (24 hour) there were differences in

269 both activity and sheltering behaviours. Overall, activity levels significantly decreased from 2.51

270 to 0 (+/- 0.034 SEM) minutes per hour at 160 dB re 1μ Pa treatment and increased from 2.51 to

271 8.36 (+/- 1.0 SEM) minutes at 170 dB re 1μ Pa, ($F_{2,12}$ =9.989, p=0.002; Fig. 6a). Post-hoc tests

272 demonstrated significant differences in activity levels between the control treatment and 170 dB

273 re 1μPa of noise (p=0.020) and between 160 and 170 dB re 1μPa (p=0.002), indicating that fish

274   were more active during longer (24 hour) exposure of 170 dB re 1μPa compared to the control

275   treatment. However, there was no significant difference in activity levels between the control and

276   160 dB re 1μPa treatment (p=0.442). Sheltering behaviour differed overall when bullhead were

277   exposed to noise ($F_{2,12}$ = 10.799, p= 0.001; Fig. 6b). Post-hoc tests indicated a non-significant

278   difference in sheltering between the control treatment and 170 dB re 1μPa (p=0.072) of noise

279   and also between control treatment and 160 dB re 1μPa (p=0.095). However, bullhead sheltered

280   more at 160 dB re 1μPa (60 minutes per hour) compared to 170 dB re 1μPa (50 minutes per

281   hour) (p=0.001). During acute exposure of both noise levels of 160 and 170 dB re 1μPa, bullhead

282   did not exhibit a change in cortisol levels compared to the control ($F_{2,14}$ = 1.305, p=0.302; Fig.

283   6c). Cortisol data collected during the chronic noise treatment uncovered no significant

284   differences ($F_{2,15}$ = 3.268, p=0.066; Fig. 6c). Post-hoc analyses revealed no significant difference

285   between cortisol levels in the no-noise control experiment compared to 160 dB re 1μPa

286   (p=0.147) and 170 dB re1μPa (p=0.992); and between the no-noise control and 170 dB re 1μPa

287   (p=0.075).

288   There was a significant effect of 160 and 170 dB re1μPa noise exposure on ciliary bundle

289   number ($F_{2,30}$ =18.458, p <0.001; Fig. 6d), resulting in fewer ciliary bundles present in noise

290   treatments than no-noise controls. Post-hoc tests further uncovered a significant difference in

291   ciliary bundles of hair cells when comparing the no-noise control and 170 dB re 1μPa treatment

292   (p<0.001) during short term acute exposure. During long term exposure of noise, post-hoc

293   analyses determined a significant difference in ciliary bundle number between the control and

294   170 dB re1μPa (p<0.001),160 vs 170 dB re 1μPa (p=0.001), however there was not a significant

295   difference between the no-noise control and 160 dB re 1μPa (p=0.062) (Fig. 6d). There was no

296   significant difference in the number of ciliary bundles present in each box placed along the

297   saccular epithelium in both control and exposed ears (*p*=0.0727), showing no regional effects of

298   sound exposure on hair cell damage (Fig. 4).

299   **Discussion**

300   Anthropogenic noise caused a change in behavioural characteristics and ciliary bundles in black

301   bullhead. Bullhead exhibited an increase in sheltering behaviour and a decrease in activity levels

302   even when exposed to 140 dB re1µPa and had fewer ciliary bundles when exposed to 170 dB

303   re1µPa during both short and long period acute exposure. Noise pollution research is not

304   commonly studied in freshwater environments (Mickle & Higgs 2017), even though these

305   environments are species rich and important economically and recreationally as we rely on fish

306   as a major source of protein for the world's population (16%) (Tidwell & Allan 2001). As such,

307   we would suggest that more resources be dedicated to better understanding possible fitness

308   effects of anthropogenic noise in these critical habitats.

309   *Behavioural Responses*

310   The decreased activity patterns demonstrated by bullhead when exposed to 140 dB re 1µPa

311   suggest this level of anthropogenic noise impacts behavioural responses which can be a

312   precursor to a physiological stress response (Eriksson & Van veen 1980; Valdimarrson &

313   Metcalfe 1998). Contrary to some literature (Smyly 1957; Lelek 1987), bullhead are not

314   normally sedentary in nature, but instead are mobile (usually under dark conditions) to detect

315   prey species and find suitable spawning habitats (Eriksson & Van veen 1980; Knaepkens et al.

316   2004). Research has indicated that fish can often exhibit avoidance behaviours (Ona & Godø

317   1990; Fewtrell & McCauley 2012) in response to noise; however, due to constraints of tank size,

318   we used activity levels to indicate a change in behavioural characteristics. McLaughlin and Kunc

319   (2015) examined the behavioural impacts of boat noise on the convict cichlid (*Amatitlania*

320   *nigrofsciata*) and found that although the presence of a boat noise increased time spent sheltering

321   and decreased spent time foraging, it did not alter their overall activity level. Activity effects in

322   bullhead may be due to the enhanced hearing capability of bullhead and suggests caution in

323   extrapolating effects between species with different hearing abilities.

324   *Physiological Responses*

325   Cortisol levels were used as a measure of physiological stress when fish were exposed to noise

326   (Donaldson 1981; Wysocki et al. 2006; Barcellos et al. 2007). While our behavioural results

327   suggested that fish were showing a stress response, this was not indicated by the physiological

328   marker of cortisol level. This apparent discrepancy can be explained by the principle that animals

329   first respond to stress through a behavioural mechanism (Dawkins 2003; Moberg & Mench

330   2005). It is also possible that there was no clear pattern associated with cortisol data due to

331   sampling at the end of the experiment. If bullhead exhibited a spike in cortisol at the beginning

332   of the experiment when fish were first introduced to the noise, it is possible cortisol levels

333   returned to baseline levels after a certain amount of time had passed. Thus, our findings do not

334   suggest that bullhead do not exhibit signs of physiological stress, to confirm this, more stress

335   markers such as: glucose, lactate, cardiac output and changes in oxidative stress or immune

336   response could be measured (Graham & Cooke 2008; Dantzer et al. 2014). Finally, physiological

337   responses are highly context specific and can be modified by a number of intrinsic and extrinsic

338   factors (Madliger & Love 2014). As a result, physiological stress and changes in growth and

339   condition may only be apparent after longer time periods or repeated exposures to noise

340   stressors, and the extent of these responses may be different during different life history stages

341   (Dantzer et al. 2014; Shannon et al. 2016).

342 *Ciliary Bundle Data*

343 Based on results obtained from the first noise treatment of 140 dB re 1 μPa, we decided to

344 expose bullhead to both short and long term acute periods of 160 and 170 dB re 1μPa to

345 determine what the impacts were at these higher noise levels. As fish were being housed in

346 communal tanks, we changed the experimental design during the higher intensity noise treatment

347 to more accurately represent normal housing conditions and reduce stress in the chronic

348 treatments that necessitated holding fish for longer time. Therefore, we had 3 fish in an

349 experimental tank as opposed to 1 fish in 6 separate tanks. The boat noise played to the bullhead

350 at these higher noise levels is still ecologically relevant — small boats generally produce noise

351 ranging from 140-167 dB re 1μPa and merchant ships produce noise ranging from 178-192 dB re

352 1μPa up to an approximate distance of 2 m away (Arveson & Vendittis 2000; Amoser et al.

353 2004). Cargo ships have been shown to reach up to noise levels of 212 dB re 1μPa at 1 m away,

354 this level can create shock waves emitted from the propeller (Arveson & Vendittis 2000). During

355 long term acute exposure, fish were less active at 160 dB re 1μPa but were more active during

356 170 dB re 1μPa when compared to the control. These behavioural effects may be attributed to the

357 ciliary bundle data; if there is a decrease in ciliary bundles of hair cells after both short and long

358 term acute stressors of noise played at 170 dB re 1μPa, bullhead hearing sensitivity will likely

359 decrease (Smith et al. 2003) so they may no longer perceive the noise to be as loud and therefore

360 stressful. If fish are no longer sensitive to the noise it is likely they will no longer exhibit signs of

361 stress, explaining why bullhead exposed to 170 dB re 1μPa for 24 hours did not exhibit a change

362 in cortisol levels and were more active during this treatment, even relative to the controls which

363 still had background noise present in the holding conditions.

364   Higgs and colleagues (2002) looked at regional differences in hair cell density along 14 regions

365   of the saccular epithelium of zebrafish and only found density differences at the caudal end of

366   the epithelium. Smith and colleagues (2003) counted hair cells along 4 locations (2500 μm^2 size

367   boxes) along the saccular macula in goldfish, therefore, we focused on hair cell number along

368   three locations of the bullhead saccule. We found no significant regional differences in both the

369   controls and exposed ears. Previous research suggest topographic frequency dependent loss of

370   hair cells in fish (Furukawa & Ishii 1967), however our boat noise file featured a broad spectral

371   range (100-10,000 Hz), limiting the topographic effect of frequency specific hair cell damage.

372   *Future Considerations*

373   There are a few considerations when analyzing the current data. First, some fish have higher

374   baseline cortisol levels than others, which can cause variability in results. Second, cortisol levels

375   fluctuate seasonally and diurnally (Laidley & Leatherland 1988); to avoid this confounding

376   variable, all experiments were started at approximately the same time each day, over the period

377   of three months. Due to the capture of live fish, we had a large size range in bullhead, however

378   the fish were non-reproductive (fish were dissected to visualize presence of gonads) to avoid the

379   impacts reproduction could have on behaviour. As we did not test other sources of noise, we

380   cannot definitively say fish are responding to the boat noise specifically, however, we can

381   conclude that bullhead display behavioural changes and fewer ciliary bundles when exposed to

382   noise. As our research includes intensities of noise at 160 and 170 dB re 1μPa, we need to

383   determine the frequency of these noise levels in fishes environment. Most research involving

384   soundscape data is carried out in marine environments (McWilliam & Hawkins 2013;

385   Staaterman et al. 2014; Erbe et al. 2015) but the data that do exist for freshwater (e.g. Amoser et

386   al. 2004; Graham & Cooke 2008) do indicate that anthropogenic noise levels in freshwater

387   ecosystems often exceed those used here..

388   Possible next steps for future study would be to measure sound levels in local areas

389   across areas such as the Great Lakes impacted by boat noise to determine the source, timing and

390   duration of noise levels. Further research is also needed to determine boat noise impacts on

391   freshwater fish with general hearing capabilities. Another component to consider is that the fish

392   were kept in captivity and could not escape, therefore in the wild fish may simply leave the area

393   to avoid the noise. However, depending on the noise source, health status of the animal and how

394   loud it is, this may not always be possible. Recommendations to decrease noise impacts on

395   freshwater habitats include: the addition of protected areas, restricting human access to specific

396   sites (particularly spawning grounds for endangered fish), the use of physical barriers to noise

397   and widespread quiet technology (Shannon et al. 2015). Noise pollution research is not

398   commonly studied in freshwater environments (Mickle & Higgs 2017), even though these

399   environments are species rich and important for human survival (Tidwell & Allan 2001). More

400   focus should be given to noise impacts on freshwater environments to be able to truly assess the

401   impact of anthropogenic stressors on survival and fitness of these key species.

402

403

404

405

406

References:

Aalbers, S.A., and Drawbridge, M.A. 2008. White seabass spawning behavior and sound
production. Trans. Am. Fish. Soc. **137**(2)**:** 542–550. doi: 10.1577/T04-058.1.

Amoser, S., Wysocki, L.E., and Ladich, F. 2004. Noise emission during the first powerboat race
in an Alpine lake and potential impact on fish communities. J. Acoust. Soc. Am. **116**(6): 3789-
3797. doi: 10.1121/1.1808219.

Arveson, P.T., and Vendittis, D.J. 2000. Radiated noise characteristics of a modern cargo ship. J.
Acoust. Soc. Am. **107**(1): 118-129. doi: 10.1121/1.428344.

Barcellos, L.G.P., Kreutz, L., Quevedo, R.M., Bolognesi, da Silva. L., Bedin, A.C., Finco, J.,
and Cericato, L. 2007. Whole-body cortisol increases after direct and visual contact with a
predator in zebrafish, *Danio rerio*. Aquacul. **272**(1-4): 774-778.
doi:10.1016/j.aquaculture.2007.09.002.

Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes
in circulating corticosteroids. Integr. Comp. Biol. **42**(3): 517–525. doi: 10.1093/icb/42.3.517.

Bruintjes, R., and Radford, A, N. 2013. Context-dependent impacts of anthropogenic noise on
individual and social behaviour in a cooperatively breeding fish. Anim. Behav. **85**(6): 1343-
1349. doi:10.1016/j.anbehav.2013.03.025.

Cooke, S.J., Blumstein, D.T., Buchholz, R., Caro, T., Fernández-Juricic, E., Franklin, C.E.,
Metcalfe. J, O'Connor, C.M, St. Clair, C.C., Sutherland, W.J., and Wikelski, M. 2014.
Physiology, Behaviour, and Conservation. Physiol. Biochem. Zool. **87**(1): 1- 14.
doi:10.1086/671165.

440 Danzter, B., Fletcher, Q.E., Boonstra, R., and Sheriff, M.J. 2014. Measures of physiological
441 stress: a transparent or opaque window into the status, management and conservation of species?
442 Conserv. Physiol. **2**(1): 1-18. doi: 10.1093/conphys/cou023.
443
444 Dawkins, M.S. 2003. Behaviour as a tool in the assessment of animal
445 welfare. Zool. **106**(4): 383–387. doi: 10.1078/0944-2006-00122.
446
447 Donaldson, E. 1981. The pituitary-interrenal axis as an indicator of stress in fish. *In* Stress in
       Fish. *Edited by* A.D. Pickering. London, Academic. pp.11-47.
448 Dyndo, M., Wisniewska, D.M., Rojano-Donate, L. and Madsen, P.T. 2015. Harbour porpoises
449 react to low levels of high frequency vessel noise. Sci. Rep. **5:**1-9. doi:10.1038/srep11083.
450 Enger, P. 1981. Frequency discrimination in teleosts-central or peripheral? *In* Hearing and Sound
451 Communication in Fishes. Edited by W.N. Tavolga, A.N, Popper and R.R. Fay. New York,
452 Springer Verlag. pp. 243-255.
453
454 Erbe, C., Verma, A., McCauley, R., Gavrilov, A., and Parnum, I. 2015. The marine soundscape
455 of the Perth Canyon. Prog. Oceanogr. **137**: 38-51. doi: 10.1016/j.pocean.2015.05.015.
456
457 Eriksson, L.O., and Van Veen, T. 1980. Circadian rhythms in the brown bullhead, *Ictalurus*
458 *nebulosus* (Teleostei). Evidence for an endogenous rhythm in feeding, locomotor, and reaction
459 time behaviour. Can. J. Zool. **58**(10): 1899-1907. doi: 10.1139/z80-259.
460 Fay, R. (2009). Soundscapes and the sense of hearing of fishes. Integr. Zool. **4**(1), 26-32. doi:
461 10.1111/j.1749-4877.2008.00132.x.
462 Fewtrell, J.L., and McCauley, R.D. 2012. Impact of air gun noise on the behaviour of marine fish
463 and squid. Mar. Pollut. Bull. **64**(5): 984-993. doi: 10.1016/j.marpolbul.2012.02.009.
464
465 Furukawa, T., & Ishii, Y. 1967. Neurophysiological studies on hearing in goldfish. J.
466 Neurophysiol. **30**(6): 1377-1403. doi:10.1152/jn.1967.30.6.1377.
467 Graham, A.L., and Cooke, S.J. 2008. The effects of noise disturbance from various recreational
468 boating activities common to inland waters on the cardiac physiology of a freshwater fish, the
469 largemouth bass (*Micropterus salmoides*). Aquat. Conserv. **18**(7): 1315-1324.
470 doi: 10.1002/aqc.941.
471
472 Hastings, M.C., Popper, A.N., Finneran, J.J., and Lanford, P.J. 1996. Effects of low-frequency
473 underwater sound on hair cells of the inner ear and lateral line of the teleost fish *Astronotus*
474 *ocellatus*. *J*. Acoust. Soc. Am. **99**(3): 1759-1766. doi:10.1121/1.414699.
475
476 Hawkins, A. D. (1981). The hearing abilities of fish. In Hearing and sound communication in
477 fishes (pp. 109-137). Springer, New York, NY.
478
479 Higgs, D.M., Souza, M., Wilkins, H.R., Presson, J.C. and Popper, A.N. 2002. Age-and size-
480 related changes in the inner ear and hearing ability of the adult zebrafish (*Danio rerio*). JARO
481 **3**(2): 174-184.doi:10.1007/s101620020035.
482

483   Higgs, D.M., Z. Lui, D.A. Mann. (2006) Hearing and Mechanoreception. In: Evans, D.H. (ed.)
484   The Physiology of Fishes (3rd Ed.). CRC Press, pp. 391-429.

485

486   Jenkins, D. B. 1977. A light microscopic study of the saccule and lagena in certain
487   catfishes. Am. J. Anat. **150**(4): 605-629. doi:10.1002/aja.1001500407.

488

489   Kasumyan, A.O. 2005. Structure and Function of the Auditory System in Fishes. J. Ichthyol.
490   45(Suppl2): 223–270.

491

492   Knaepkens, G., Bruyndoncx, L., and Eens, M. 2004. Assessment of residency and movement of
493   the endangered bullhead (*Cottus gobio*) in two Flemish rivers. Ecol. Freshw. Fish. **13**(4): 317–
494   322. doi: 10.1111/j.1600-0633.2004.00065.x.

495

496   Ladich, F., and Fay, R.R. 2013. Auditory evoked potential audiometry in fish. Rev. Fish Biol.
497   **23**(3): 317–364. doi: 10.1007/s11160-012-9297-z.

498

499   Laidley, C.W., and Leatherland, J.F. 1988. Circadian studies of plasma cortisol, protein, glucose
500   and ion concentration, liver glycogen concentration and liver and spleen weight in rainbow trout,
501   *Salmo gairdneri* Richardson. Comp. Biochem. Physiol. **89**(3): 495-502. doi:10.1016/0300-
502   9629(88)91063-8.

503

504   Lelek, A. 1987. The freshwater fishes of Europe: Threatened fishes of Europe. Wiesbaden: Aula-
505   Verlag.

506   Madliger, C. L., and Love, O. P. 2014. The Need for a Predictive, Context-Dependent Approach
507   to the Application of Stress Hormones in Conservation. Conserv. Biol. **28**(1): 283–287.
508   doi:10.1111/cobi.12185.

509

510   McLaughlin, K.E., and Kunc, H.P. 2015. Changes in the Acoustic Environment Alter the
511   Foraging and Sheltering Behaviour of the Cichlid *Amititlania nigrofasciata*. Behav. Processes.
512   **116**: 75-79. doi:10.1016/j.beproc.2015.04.012.

513

514   McWilliam, J.N., and Hawkins, A.D. 2013. A comparison of inshore marine soundscapes. J.
515   Exp. Mar. Biol. Ecol. **446**:166-176. doi: 10.1016/j.jembe.2013.05.012.

516

517   Mickle, M.F., and Higgs, D.M. 2017. Integrating techniques: A review of the effects of
518   anthropogenic noise on freshwater fish. Can. J. Fish Aq. Sc. **999**:1-8. doi:10.1139/cjfas-2017-
519   0245.

520

521   Mommsen, T.P., Vijayan, M.M., and Moon, T.W. 1999. Cortisol in teleosts: Dynamics,
522   mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. **9**(3): 211–268.
523   doi:10.3389/conf.fendo.2011.04.00092.

524

525   Moberg, G.P., and Mench, J.A. 2005. The biology of animal stress: basic principles and
526   implications for animal welfare. Wallingford: CABI publication.

527

528 Neo, Y. Y., Parie, L., Bakker, F., Snelderwaard, P., Tudorache, C., Schaaf, M., and Slabbekoorn,
529 H. 2015. Behavioral changes in response to sound exposure and no spatial avoidance of noisy
530 conditions in captive zebrafish. Front. Behav. Neuro. **9**(28): 1-11.
531 doi:10.3389/fnbeh.2015.00028.

532 Nichols, T.A., Anderson, T.W., and Širović, A. 2015. Intermittent noise induces physiological
533 stress in a coastal marine fish. PLOS ONE. **10**(9): 1-10. doi: 10.1371/journal.pone.0139157.
534
535 Ona, E. and Godø, O. (1990). Fish reaction to trawling noise: the significance for trawl sampling.
536 *Rapp. P.-v. Réun*. **189**: 159-166.
537
538 Parvulescue, A. (ed). 1964. Problems of propagation and processing. Marine bio-acoustics.
539 Pergamon Press, Oxford.
540
541 Payne, N.L., van der Meulen, D., Suthers, I.M., Gray, C.A., and Taylor, M.D. 2014. Foraging
542 intensity of wild mulloway *Argyrsomus Japonicus* decreases with increasing anthropogenic
543 disturbance. Mar. Biol. **162**(3): 539-546. doi:10.1007/s00227-014-2603-7.
544
545 Poggendorf, D. 1952. Die absolute Hörschwelle des Zwergwelses (*Amiurus nebulosus*) und
546 Beiträge zur Physik des Weberschen Apparates der Ostariophysen. *Z.* Vergl. Physiol. **34**(3):
547 222–257. doi:10.1007/bf00298202.

548 Popper, A.N. 2003. Effects of anthropogenic sounds on fish. Fisheries. **28**(10): 24-31.
549 doi:10.1577/1548-8446(2003)28[24:EOASOF]2.0.CO;2.

550 Popper, A.N., and Hastings, M.C. 2009. The effects of anthropogenic sources of sound on fishes.
551 J. Fish Biol. **75**(3): 455–489. doi:10.1111/j.1095-8649.2009.02319.x.
552
553 Popper, A.N. and Hawkins, A. 2012. The effects of noise on aquatic life. New York: Springer.
554
555 Rogers, P.H., A.D. Hawkins, Popper, A.N., Fay, R.R., and Gray, M.D. 1995. Parvulescu
556 revisted: small tank acoustics for bioacousticians. In: Popper, A.N. and Hawkins, A.D. (eds). The
557 Effects of Noise on Aquatic Noise II. Springer-Verlag, NY, pp. 933-941.
558
559 Ross, D. 1976. Mechanics of Underwater Noise. Pergamon Press: New York.

560 Sabet, S.S., Neo Y.Y., and Slabbekoorn, H. 2015. The effect of temporal variation in sound
561 exposure on swimming and foraging behaviour of captive zebrafish. Anim. Behav. **107**:49-60.
562 doi:10.1016/j.anbehav.2015.05.022.

563 Shannon, G., Mckenna, M.F., Angeloni, L.M., Crooks, K.R., Fristrup, K.M., Brown, E., Warner,
564 K.A., Nelson, M.D., White, C., Briggs, J., McFarland, S Wittemyer, G. 2015. A synthesis of two
565 decades of research documenting the effects of noise on wildlife. Biol. Rev. **91**(4): 982-
566 1005. doi:10.1111/brv.12207.

567 Simpson, S.D., Radford, A.N., Nedelec, S.L., Ferrari, MCO., Chivers, D.P., McCormick, M.I.,
568 and Meekan, M.G. 2016. Anthropogenic noise increases fish mortality by predation. Nat.
569 Commun. **7**: 1-6. doi: 10.1038/ncomms10544.

570 Slabbekoorn, H., Bouton, N., Opzeeland, I., Coers, A., Cate, C.,, and Popper, A.N. 2010. A noisy
571 spring: the impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. **25**(7):
572 419-427. doi:10.1016/j.tree.2010.04.005.

574 Smith, M.E., Kane, A.S., and Popper, A.N. 2003. Noise-induced stress response and hearing loss
575 in goldfish (*Carassius Auratus*). J. Exp. Bio. **207**(3): 427-435. doi:10.1242/jeb.00755.

577 Smith, M. E., Coffin, A. B., Miller, D. L., and Popper, A. N. (2006). Anatomical and functional
578 recovery of the goldfish (*Carassius auratus*) ear following noise exposure. J. Exp. Bio. **209**(21):
579 4193-4202. doi: 10.1242/jeb.02490

580 Smyly, W.J.P. 1957. The life-history of the bullhead or Miller's thumb (*Cottus gobio*). Proc.
581 Zool. Soc. Lon. **128**(3): 431–453. doi:10.1111/j.1096-3642.1957.tb00336.x.

582 Solan, M., Hauton, C., Godbold, J.A., Wood, C.L., Leighton, T.G., and White, P. 2016.
583 Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates
584 mediate ecosystem properties. Sci. Rep. **6**: 1-9. doi: 10.1038/srep20540.

586 Staaterman, E., Paris, C.B., DeFerrari, H.A., Mann, D.A., Rice, A.N., and D'Alessandro, E.K.
587 2014. Celestial patterns in marine soundscapes. Mar. Ecol. Prog. Ser. **508**: 17-32.
588 doi:10.3354/meps10911.

590 Tidwell, J.H., and Allan, G.L. 2001. Fish as food: aquacultures contribution. EMBO Rep. **2**(11):
591 958-963. doi:10.1093/embo-reports/kve236.

593 Valdimarrson, S.K., and Metcalfe, N.B. 1998. Shelter selection in juvenile Atlantic salmon, or
594 why do salmon seek shelter in winter? J. Fish Biol. **52**(1): 42-49. doi:10.1006/jfbi.1997.0557.

595 Wale, M.A., Simpson, S.D., and Radford, A.N. 2013. Noise negatively affects foraging and
596 antipredator behaviour in shore crabs. Anim. Behav. **86**(1): 111-118.
597 doi:10.1016/j.anbehav.2013.05.001.

598 Wysocki, L. E., and Ladich, F. 2005. Effects of noise exposure on click detection and the
599 temporal resolution ability of the goldfish auditory system. Hear. Res. **201**(1-2): 27-36.
600 doi:10.1016/j.heares.2004.08.015.

601 Wysocki, L.E., Dittami, J.P. and Ladich, F. 2006. Ship Noise and Cortisol Secretion in European
602 Freshwater Fishes. Biol. Conserv. **128** (4): 501-508. doi:10.1016/j.biocon.2005.10.020.

604 Wysocki, L.E., Davidon, J.W., Smith, M.E., Frankel, A.S., Ellison, W.T., Mazik, P.M., Popper,
605 A.N., and Bebak, J. 2007. Effects of aquaculture production noise on hearing, growth, and
606 disease resistance of rainbow trout *Oncorhynchus mykiss*. Aquaculture. **272**(1-4): 687-697. doi:
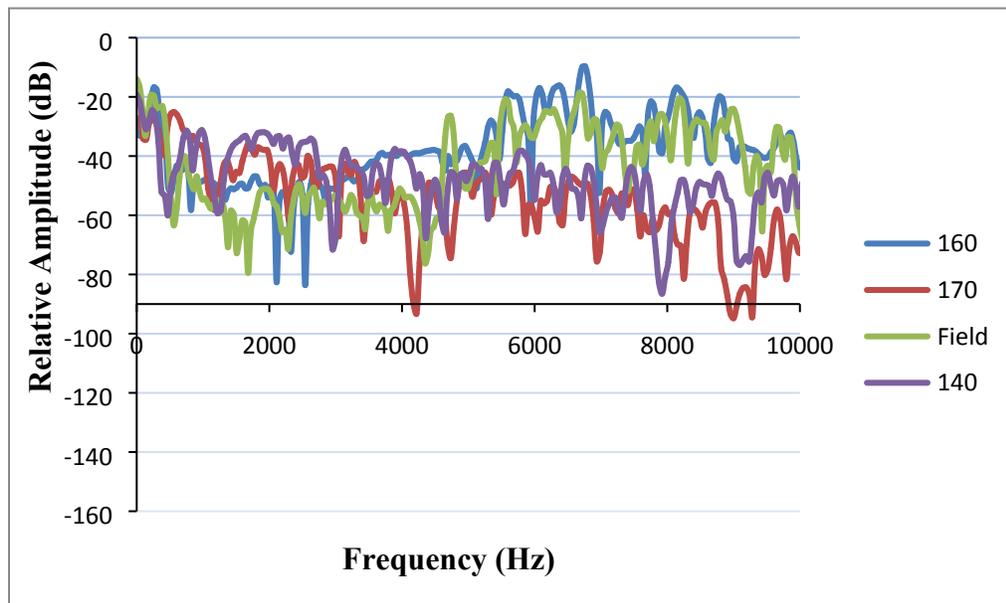607 10.1016/j.aquaculture.2007.07.225.

Figure1: A spectogram comparing the output of the boat noise file from the field and a hydrophone recording of the noise file collected from the experimental tank when played at 140, 160 and 170 dB re 1 μPa.
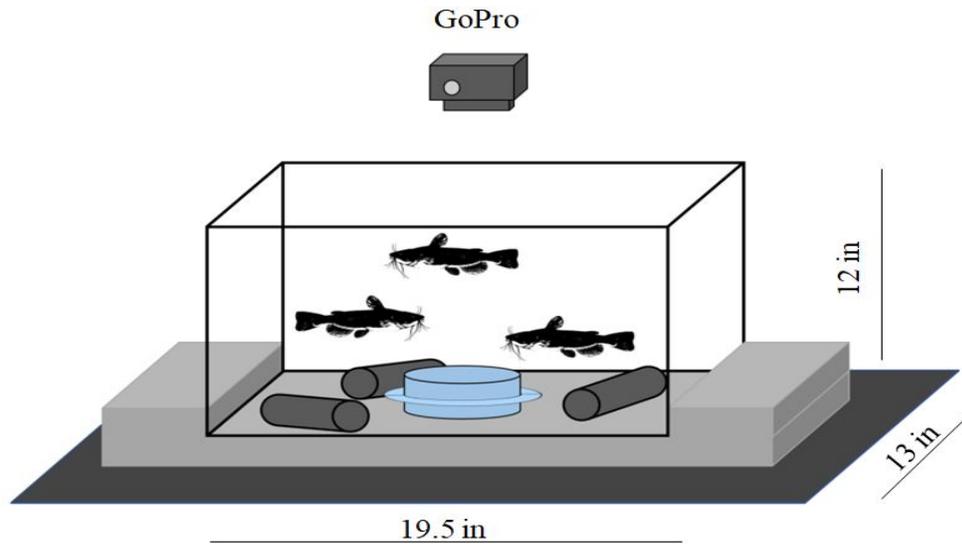
Figure 2: The experimental tank set-up used in both the 140 dB re 1 μPa and 160/170 dB re 1 μPa trials (although only one fish was placed in this tank during the 140 dB re 1 μPa trial). The test tanks were fitted into a Styrofoam holding structure and placed on top of two acetal plastic sheets, 2 cm thick, to minimize acoustic disturbance and vibrations from the floor.
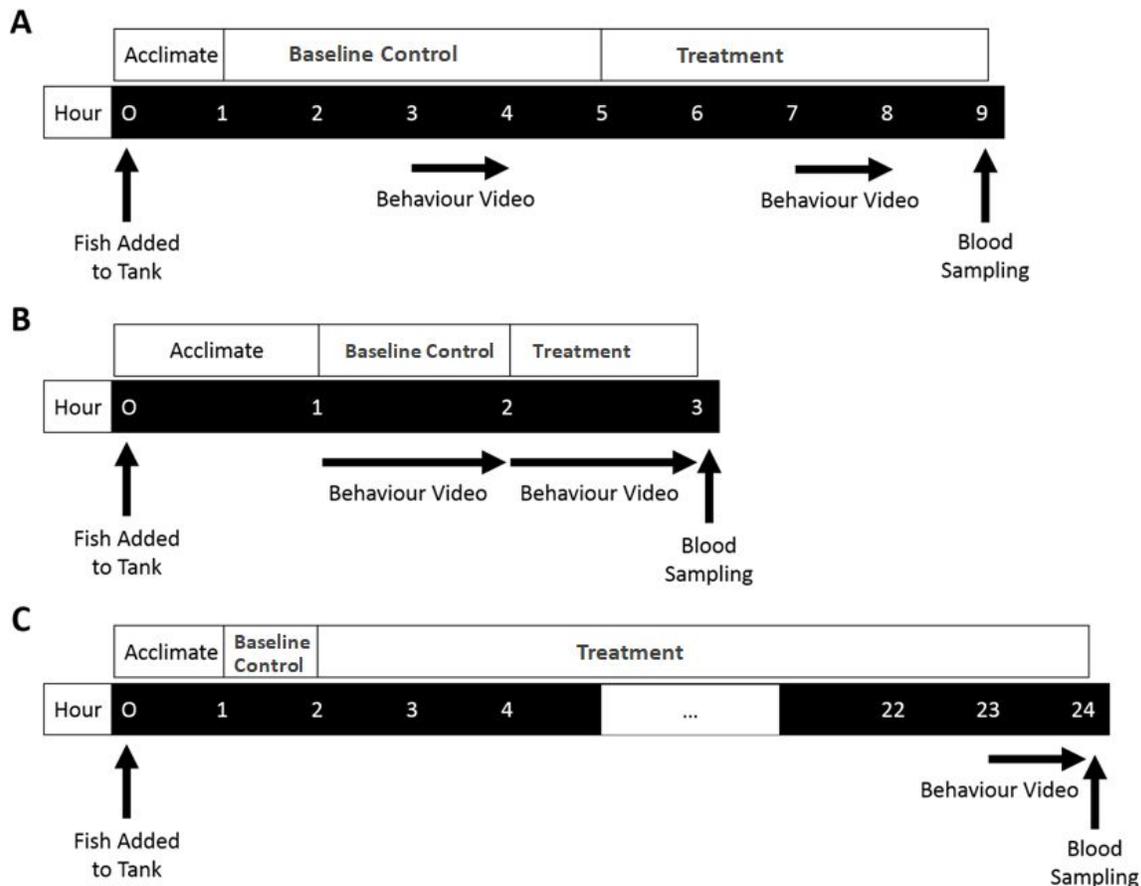
Figure 3:

a) Six bullhead were placed in separate experimental tanks during the 140 dB re 1 µPa exposure experiment. Bullhead acclimated for one hour, after which time a four hour baseline control trial took place followed by a four hour treatment (either noise or silence). Bullhead were anesthetized and blood sampling took place immediately following the experiment.

b) Three bullhead were added into one experimental tank during the higher intensity (160 or 170 dB re 1 µPa) short-term acute noise experiment. Bullhead acclimated for one hour, followed by a one hour baseline control trial and one hour treatment (either noise or silence). Bullhead were anesthetized and blood sampling took place immediately following the experiment.

c) Three bullhead were added into one experimental tank during the high intensity (160 or 170 dB re 1 µPa) long-term acute noise experiment. Bullhead acclimated for one hour, followed by a one hour baseline control trial and a twenty-four hour treatment (either noise or silence). Bullhead were anesthetized and blood sampling took place immediately following the experiment.
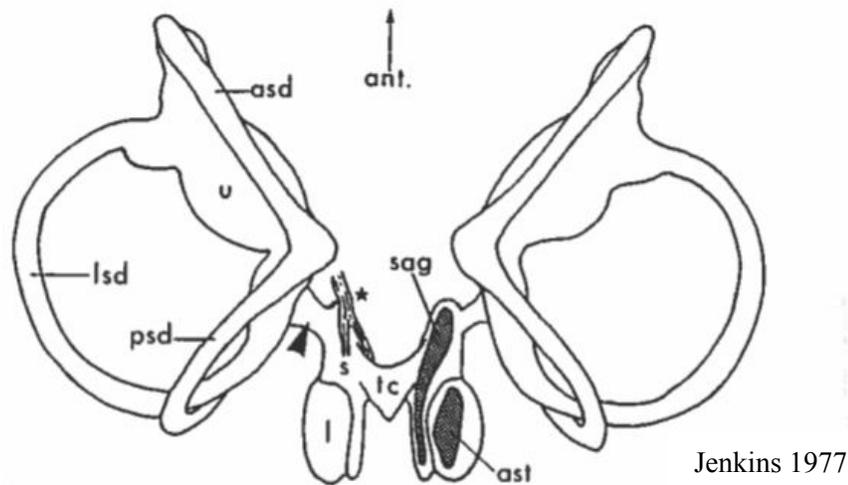
A)

B)

C)

Jenkins 1977
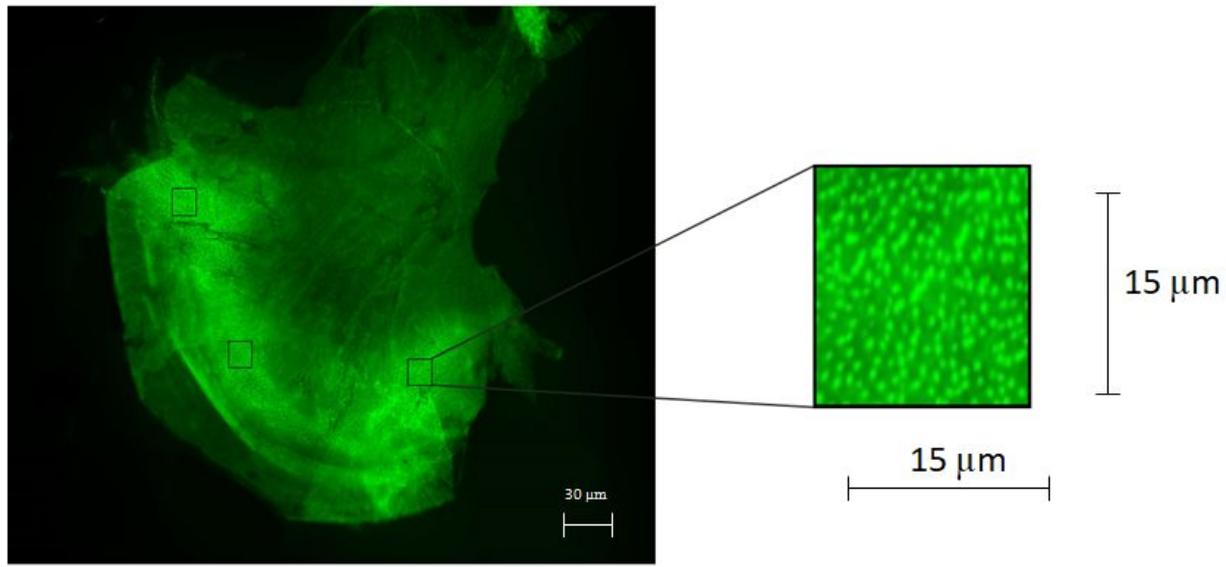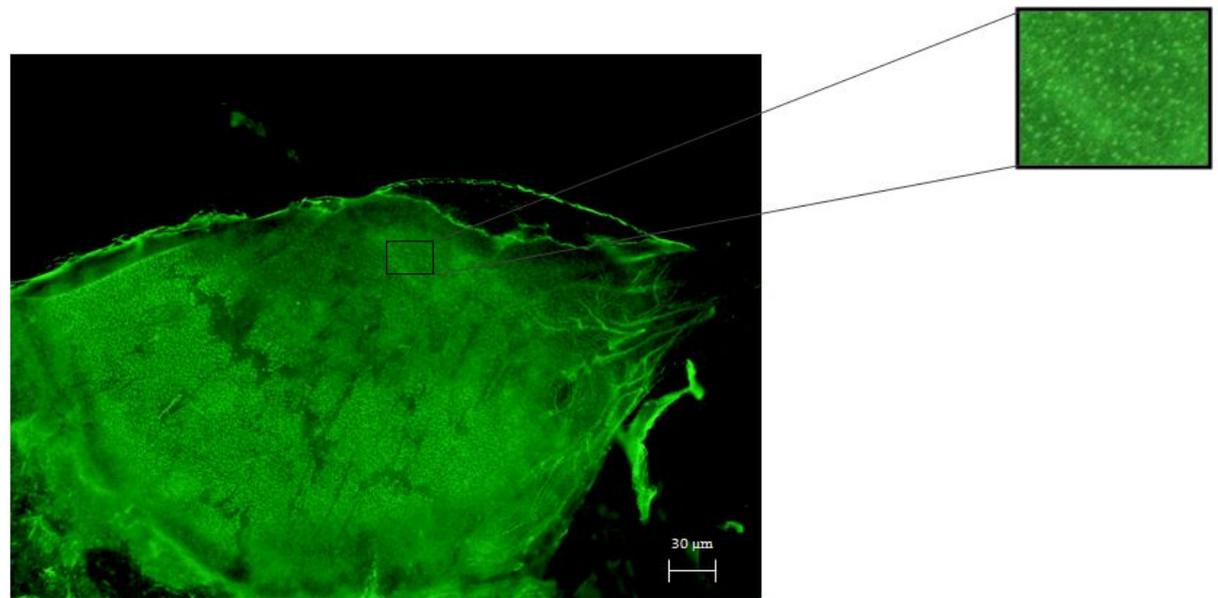
Figure 4:

 a) A dissected bullhead ear is shown in this image, exposing the saccule, which is further sectioned into three identical boxes (15 μm^2 in size in magnified counting view) to allow for hair cell counts.

b) A stained lagena in the bullhead ear is shown here to allow for comparison to the saccule. The hair cells in the lagena were not quantified.

c) A schematic of a catfish ear from Jenkins (1977) is referenced here as we used this diagram to help us identify the ear organs (u= utricle; l=lagena; s=saccule; sag= sagitta (saccular otolith)).
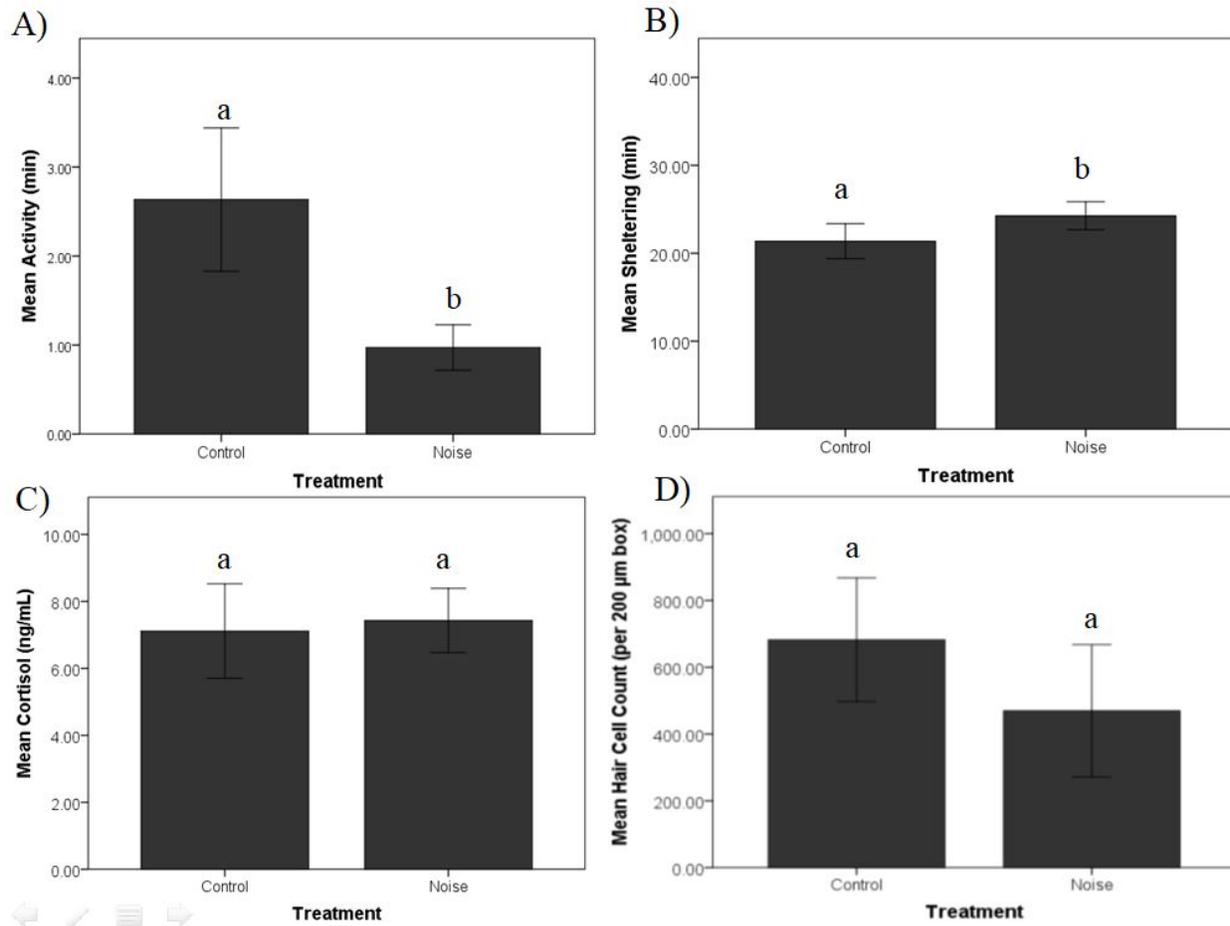
Figure 5:

a) Mean activity levels of black bullhead during exposure to 140 dB re 1μPa of boat noise and during control trials. Significant differences are indicated by different letters at the p=0.05 level, error bars are representative of mean (+/- S.E).

b) Mean levels of sheltering behaviour in black bullhead when exposed to 140 dB re 1μPa of boat noise and during control trials. Significant differences are indicated by different letters at the p=0.05 level, error bars are representative of mean (+/- S.E).

c) Mean log cortisol levels of black bullhead after exposure to 140 dB re 1 μ Pa of boat noise compared to controls. Significant differences are indicated by different letters at the p=0.05 level, error bars are representative of mean (+/- S.E).

d) Mean hair cell counts of black bullhead when exposed to 140 dB re 1μPa of boat noise and in control trials. Significant differences are indicated by different letters at the p=0.05 level. Error bars are representative of mean (+/- S.E).
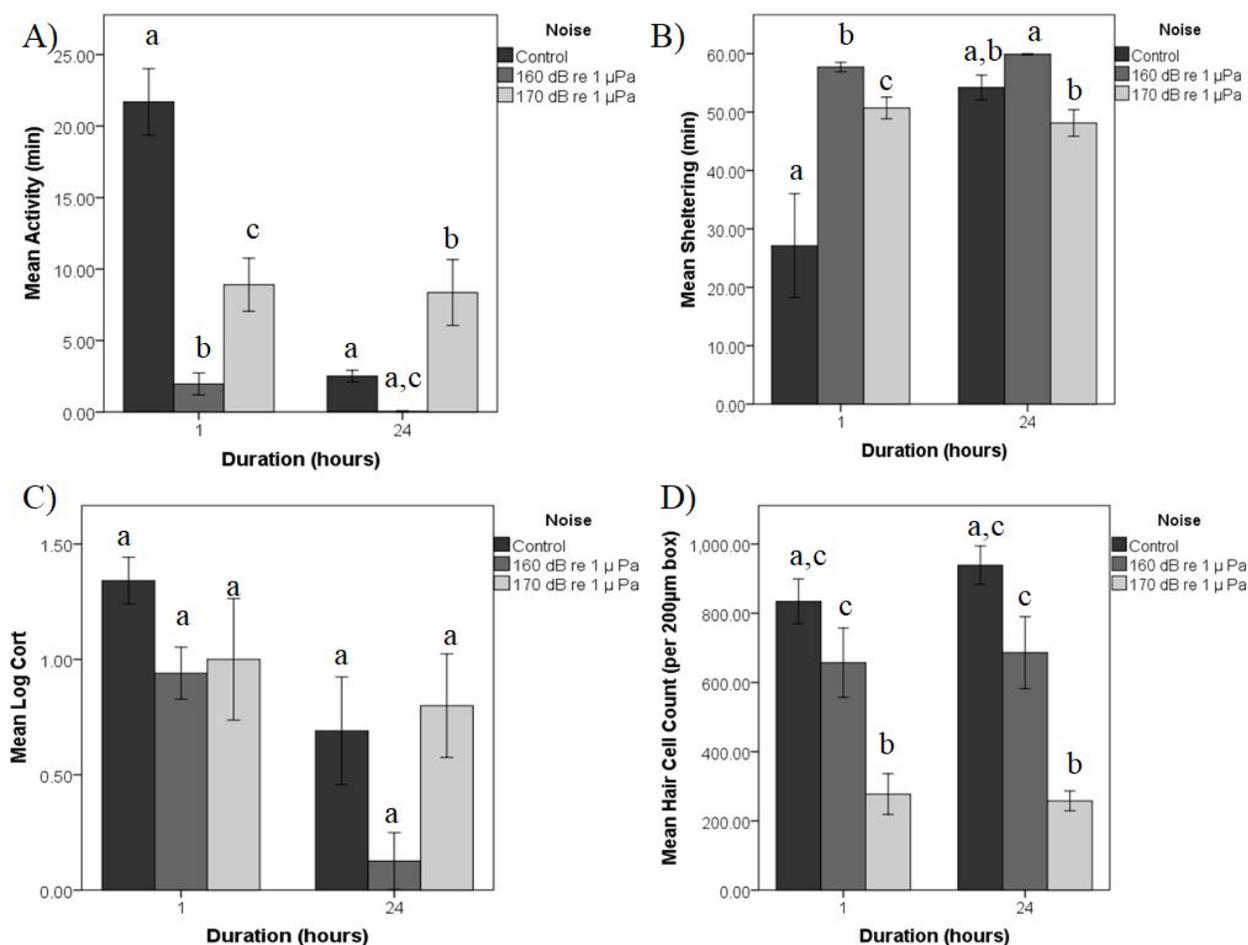
Figure 6:

a) Mean activity levels of bullhead when exposed to 160 and 170 dB re 1μPa of boat noise at two time points. Significant differences are indicated by different letters at the $p=0.05$ level. Error bars are representative of mean (+/- S.E).

b) Mean sheltering levels of bullhead when exposed to 160 and 170 dB re 1μPa of boat noise at two time points. Significant differences are indicated by different letters at the $p=0.05$ level. Error bars are representative of mean (+/- S.E).

c) Mean cortisol levels of bullhead when exposed to 160 and 170 dB re 1μPa of boat noise at two time points. Significant differences are indicated by different letters at the $p=0.05$ level. Error bars are representative of mean (+/- S.E).

d) Mean hair cell counts of bullhead when exposed to 160 and 170 dB re 1μPa of boat noise at two time points. Significant differences are indicated by different letters at the $p=0.05$ level. Error bars are representative of mean (+/- S.E).