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

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Abstract: There is increasing concern about the effect of underwater noise on fish due to rising levels of anthropogenic noise. We performed experiments on the black bullhead (*Ameiurus melas*), a species with known hearing specializations and located within the Laurentian Great Lakes where there is considerable commercial and recreational boat traffic. We tested and compared physiology (baseline cortisol), behaviour (activity, sheltering), and morphology (ciliary bundles of hair cells) of bullhead to boat noise. At 140 dB re 1 μ Pa (-54.84 dB re 1 m·s⁻²), we saw clear behavioural effects in terms of both activity and sheltering levels despite no obvious morphological or physiological stress. Following both short- and long-period acute exposure to higher — but environmentally relevant — noise levels, bullhead were less active and sheltered more and also exhibited a decrease in ciliary bundles. These results suggest that there are sublethal effects of anthropogenic noise on fish behaviour and ciliary bundles, which may have direct implications on population health. Moreover, commonly used metrics such as stress hormones may not always offer the most relevant biomarker of the response to anthropogenic boat noise.

Résumé : L'effet du bruit sous l'eau sur les poissons suscite de plus en plus d'inquiétudes en raison des niveaux croissants de bruit d'origine humaine. Nous avons réalisé des expériences sur la barbotte noire (*Ameiurus melas*), une espèce montrant des spécialisations auditives présente dans les Grands Lacs laurentiens, le lieu d'une importante circulation d'embarcations commerciales et récréatives. Nous avons vérifié et comparé la physiologie (concentration de base de cortisol), le comportement (activité, mise à l'abri) et la morphologie (touffes ciliaires de cellules ciliées) de barbottes noires en présence de bruit d'embarcation. À 140 dB re 1 μ Pa (–54,84 dB re 1 m·s⁻²), nous avons noté des effets comportementaux clairs en ce qui concerne les niveaux d'activité et de mise à l'abri, malgré l'absence de stress morphologique ou physiologique évident. Après des périodes d'exposition aiguë courtes et longues à des niveaux de bruit plus élevés, mais toujours pertinents sur le plan environnemental, les barbottes noires étaient moins actives et s'abritaient plus, en plus de présenter une diminution des touffes ciliaires. Ces résultats donnent à penser que le bruit d'origine humaine a des effets sublétaux sur le comportement des poissons et les touffes ciliaires, qui pourraient avoir des conséquences directes sur la santé des populations. En outre, des paramètres couramment utilisés comme la concentration d'hormones de stress pourraient ne pas toujours être les biomarqueurs les plus pertinents de la réaction au bruit d'embarcations. [Traduit par la Rédaction]

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 and 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 and 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. 2016), 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. 2016; 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 and 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 nonlethal behavioural and physiological stressors to death (Weilgart 2007; Mickle and Higgs 2018). Research that has been performed regarding noise impacts on freshwater fish is generally focused on aquaria fish such as goldfish (*Carassius auratus*; Wysocki and Ladich 2005; Smith et al. 2004), zebrafish (*Danio rerio*; Neo et al. 2015; Sabet et al. 2015), and cichlids (Cichlidae; Hastings et al. 1996; Bruintjes and 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 there have been comparatively fewer studies on effects of anthropogenic noise. Given the importance of both freshwater lakes and fish to shipping and the economy, more research is also needed to determine the full impact of shipping noise in these high traffic environments.

Hearing is an important sensory modality in fish for communication and orientation, with a great diversity in hearing abilities among species (Hawkins 1981; Aalbers and Drawbridge 2008; Fay 2009). To date, the majority of research regarding noise pollution incorporates either behavioural or physiological measures alone, but seldom have single studies integrated techniques (Cooke et al. 2014; Mickle and Higgs 2018). Previous work on fishes leads to the

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suggestion that some individuals exposed to anthropogenic noise show physiological stress responses such as increased levels of stress hormones (Barcellos et al. 2007) and change in cardiac output (Graham and Cooke 2008) and further hypothesizes changes in gene expression and immune function (Mommsen et al. 1999; Barton 2002; Shannon et al. 2016). Fish exposed to loud sounds may also suffer from physical impairments such as hair cell damage (Hastings et al. 1996; Wysocki et al. 2007) or a shift in their hearing threshold (Enger 1981; Smith et al. 2006). Finally, behavioural changes have also been seen in response to loud sounds with changes in overall behaviour level (Ona and Godø 1990), feeding behaviour (Payne et al. 2014), and predator-prey interactions (Sabet et al. 2015; Simpson et al. 2016). While these individual effects are informative, more integrative work may more accurately identify possible noise effects on fish. Thus, increased efforts on integrative studies in freshwater fish will help to better understand possible effects on anthropogenic noise in aquatic environments.

Here we take an integrative approach to examine potential impacts of noise on the phenotypic responses of black bullhead (Ameiurus melas), a species hypothesized to have specialized hearing capabilities based on previous research on Siluriformes (Poggendorf 1952; Kleerekoper and Roggenkamp 1959; Lechner and Ladich 2008) and located within the Laurentian Great Lakes where there is considerable commercial and recreational boat traffic. To obtain a holistic measure of the phenotypic response to noise in this species, we measured behavioural, physiological, and morphological changes across biologically relevant noise levels. First, we examined the impacts of noise levels at 140 dB re 1 µPa root mean square (RMS; ranging in frequency from 100 to 10 000 Hz) on bullhead behaviour and physiology, and then to further explore these results we exposed fish to differing intensities of noise (160 and 170 dB re 1 µPa RMS; 100-10 000 Hz) across two time points, 1 and 24 h. We hypothesized that bullhead exposed to 140 dB re 1 µPa would exhibit behavioural changes, while bullhead exposed to 160 and 170 dB re 1 µPa would exhibit changes in behaviour, physiology, and morphology in response to noise. More specifically, we hypothesized that bullhead under acute noise exposure would exhibit behavioural and physiological responses to noise, while those with chronic exposure will exhibit physical damage to hearing-related tissues.

Methods

Experimental design

All work was conducted under approved Canadian Council for Animal Care protocols (University of Windsor AUPP 14-11). Nonreproductive black bullhead, ranging from 22.02 to 117.60 g were obtained from a fish farm in Harrow, Essex County, Ontario (42°01′14.5″N, 83°00′04.1″W). Owing to the constraints of catching wild or farmed fish, these species were a range in size. Fish were housed at a temperature of 22.2 °C and a pH of 6.5–7 in animal quarters at the University of Windsor. The fish were fed daily and kept at a 12 h light : 12 h dark cycle to mimic natural conditions; however, as these species prefer dark environments, all housing tanks were covered with black garbage bags and opaque tank covers.

Noise levels were collected from housing and experimental tanks using a hydrophone system (InterOcean Systems, Acoustic Calibration and System Model 902) and, where possible, using a waterproofed accelerometer (model 4524 cubic triaxial deltatron, Brüel & Kjær) to estimate noise levels as pressure and particle motion. Both the hydrophone and the accelerometer were suspended in the middle of the tank, and both were used because the fish ear is fundamentally responsive to acceleration but pressure measurements are more easily understood for those concerned about noise exposure in a field setting (see Hawkins and Popper 2018). We also recognize the inherent problems with complex

Fig. 1. A spectrogram 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 μ Pa.



acoustics in small tanks (Parvulescu 1964; Rogers et al. 1995), but the current set of experiments would not be possible in a field setting and can still provide useful information about possible noise effects. The background noise in the experimental tanks was below 120 dB re 1 μ Pa RMS and below the noise floor of the accelerometer system. Bullhead likely detect both pressure and particle components of sound, as can other ostariophysan fishes (Higgs et al. 2006), but because we ultimately want to refer these results to levels experienced in the natural habitats where anthropogenic sound is most relevant and where particle motion is still difficult to accurately measure, more emphasis is placed on the pressure units.

Two noise experiments were conducted, the first consisted of 140 dB re 1 μ Pa treatment and the second consisted of a 160 or 170 dB re 1 μ Pa noise treatment; however, both treatments played the same boat noise file (Fig. 1). Boat noise was recorded from a recreational vessel using a hydrophone (Loggerhead Instruments, model No. HTI-96-Min/3V/Exp/LED) placed at a depth of approximately 2 m and at a distance of 4 m from a boat launch in a local Great Lakes habitat (Chewitt Bay, Ontario, Canada). This sound file was then played through an underwater speaker (UW-30, Lubell Labs) at 140 (equivalent to -54.84 dB re 1 m·s⁻²), 160 (equivalent to -46.55 dB re 1 m·s⁻²) (all sound levels are in RMS; Fig. 1). Only one recording was chosen to standardize the exposure across treatments, so we do not expect these results to necessarily reflect all boat recordings possible (see Slabbekoorn and Bouton 2008).

The experimental setup included an underwater speaker (Electro-Voice UW-30) connected to an amplifier (Scosche SA300), a 12-volt PBS car battery for power, and an mp3 player to play the noise (Sony Walkman NWZ-E464). The speaker was placed in the middle of the tank and background noise was quantified using a hydrophone, which measured decibel levels at eight locations and two depths in the tank, and reliably ranged from 116 to 122 dB re 1 μ Pa.

During the 140 dB re 1 μ Pa level experiment, six individual bullhead (total *n* = 60), similar in size, were randomly collected from housing tanks and added into separate plastic experimental tanks (55 L) in a dark room equipped with red light, a polyvinyl chloride (PVC) shelter, an underwater speaker, and single air stone in each tank. During the high-intensity noise experiment (160 or 170 dB re 1 μ Pa), three fish (total *n* = 24) similar in size were added into one plastic experimental tank, with the same set-up as the lower-intensity experiment, however equipped with three PVC tubes so each fish could have an individual shelter (Fig. 2).

Behavioural assays

During the first experiment (140 dB re 1 μ Pa level noise exposure; *n* = 60), six bullhead were given an acclimation time of 1 h **Fig. 2.** The experimental tank set-up used in both the 140 and 160–170 dB re 1 μ Pa trials (although only one fish was placed in this tank during the 140 dB re 1 μ Pa 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. (Note: 1 inch = 2.5 cm.)



after which a baseline control treatment took place for 4 h followed by a 4 h noise treatment (Fig. 3A). There were two separate controls for this experiment: a baseline control and a nontreatment control. The baseline control took place after the acclimation period but before the noise was played (Fig. 3A), while the nontreatment control replicated entire experimental conditions without the presence of noise (Fig. 3). To quantify a change in behaviour, we recorded experiments using a GoPro Hero3+ (Go Pro). Sheltering and general swimming behaviours were analyzed and compared during the last hour of both the baseline control and noise treatments. We quantified a sheltering response when the fish were residing in PVC tubing (one tube in each tank), and activity levels were quantified as a measure of time spent swimming throughout the videos. Activity and sheltering accounted for the total behaviours observed during the experiment.

Based on the results from 140 dB re 1 µPa, we decided to perform a second experiment to observe the impacts of higher noise levels on bullhead (160 and 170 dB re 1 µPa). During the second experiment, black bullhead (n = 24) were exposed to either 160 or 170 dB re 1 µPa of boat noise for either 1 h (short-period acute noise exposure) or 24 h (long-term acute noise exposure). Three bullhead were placed in the experiment tank (as opposed to six bullhead in individual tanks as performed in the lower-intensity experiment) and allowed to acclimate for 1 h before noise treatment (at either 160 or 170 dB re 1 µPa) began. Two separate controls (baseline and nontreatment) were also implemented for this experiment. During short-period noise exposure (1 h), fish acclimated for 1 h, after which a 1 h baseline control treatment began followed by 1 h of boat noise (at either 160 or 170 dB re 1 µPa; Fig. 3B). During the long-term noise exposure (24 h), fish were also allowed to acclimate for 1 h, followed by a 1 h baseline control treatment and then 24 h of boat noise (at either 160 or 170 dB re 1 µPa; Fig. 3C). To keep consistency in behavioural videos, we recorded the fishes' behaviour during the last hour of the long-term noise experiment. There were two experimental replicates (n = 6) for both short- and long-term acute exposure experiments, at both 160 and 170 dB re 1 µPa, totalling 24 fish (Fig. 3C). We quantified sheltering response and activity levels using the same methods presented in experiment 1. Noise experiments started at approximately the same time each day to avoid diurnal differences in behaviour.

Physiological assays

At the end of each experiment, bullhead were anaesthetized using 2-phenoxy ethanol (Sigma-Aldrich: 1 mL of 2-phenoxy ethanol per 2 L of water), and each tail was removed within 2 min so that blood from the caudal artery could be collected using a heparinized capillary vial. Once blood was collected, the fish were decapitated and heads fully submerged in paraformaldehyde (4%) for preservation before further dissection of ears. Plasma was isolated via centrifugation and cortisol was subsequently extracted from the plasma using a standard ELISA protocol. Cortisol levels were determined using a commercially available enzyme immunoassay (Cayman Chemical Company, Ann Arbor, Michigan) with assays performed according to kit instructions. Before beginning assays, a pool of black bullhead plasma was assayed both raw and after dichloromethane extraction. Serial dilutions of both were found to be parallel to the standard curve. As extracted samples showed reduced values due to recovery losses and raw plasma showed no indication of interference, samples were run on raw plasma without extraction. To ensure sample cortisol values fell within the kit detection range, we assayed bullhead samples at 1:20 dilution (10 µL of plasma and 190 µL of assay buffer). Sample concentrations were determined using an eight-point standard curve run in duplicate on each assay plate. Standards ranged in concentration from 4000 to 6.6 pg·mL⁻¹, while the minimum detection limit of the assay is 35 pg·mL⁻¹. At the end of incubation, the absorbance values for each well were measured at 412 nm using a BioTek Synergy H1 plate reader. All samples were assayed across seven plates yielding an interassay variation of 19.96% and intra-assay variation of 5.3% for bullhead.

Ciliary bundle counts

The catfish saccule is an irregular structure, twice the length of the lagena with rounded anterior-posterior ends (Jenkins 1977); the saccule location in bullhead was determined based on the schematic depicted in Jenkins (1977) (Fig. 4C). Saccules were dissected (using a Leica L2 10445930 dissecting scope) from one of every six bullhead ears randomly selected from the low-intensity noise experiment (total n = 6) and from one of every three bullhead from the high-intensity noise experiment (total n = 8). After saccules were collected, they were preserved in paraformaldehyde (4%) until stained with 12.5 µL of fluorescent green phalloidin mixed with 200 µL of phosphate buffer (Higgs et al. 2002). Once saccules were properly stained, ciliary bundles of hair cells were visualized through images collected from a Leica microscope (Leica DM IRB inverted fluorescence microscope, using the software Las A.F. 4.5). As there are thousands of ciliary bundles of hair cells present along the saccular epithelium (Higgs et al. 2002), ciliary bundles were counted in three regions along the anterior, middle, and posterior saccule using a magnified view of the epithelium. Images were imported into Adobe Photoshop (version 3.0; Adobe Systems) to create three identical boxes of 225 μ m² in size (in magnified view) representing 19% of the total saccular area (Higgs et al. 2002) (Fig. 4). Ciliary bundles within each box were then counted using Image J software (NIH) (Fig. 4). Hair cell damage was characterized as a difference in absolute number of ciliary bundles between fish exposed to noise and control fish. Comparisons in ciliary bundles of hair cell number were made between bullhead in the no-treatment control and sound exposure experiments.

Statistical analyses

Both controls (baseline and no-noise treatment) in the two experiments yielded similar behavioural results; therefore, for the purposes of this study, statistics are only reported for comparisons between the baseline control and noise exposure treatments. There was no difference in activity levels between the acclimation period and the no-noise control period; thus, time of residence in the tank was accounted for. Once data were collected, a one-way analysis of variance (ANOVA), designating fish ID as a random factor, was performed using SPSS (IBM Corp.) to analyze behavioural differences of black bullhead when exposed to noise. Ciliary bundle data were analyzed using an independent sample *t* test

Fig. 3. (A) Six bullhead were placed in separate experimental tanks during the 140 dB re 1 μ Pa exposure experiment. Bullhead acclimated for 1 h, after which time a 4 h baseline control trial took place followed by a 4 h 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 μ Pa) short-term acute noise experiment. Bullhead acclimated for 1 h, followed by a 1 h baseline control trial and 1 h 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 μ Pa) long-term acute noise experiment. Bullhead acclimated for 1 h, followed by a 1 h baseline control trial and a 24 h treatment (either noise or silence). Bullhead were anesthetized and blood sampling took place immediately following the experiment.



between no-noise controls and noise exposures, designating hair cell position as a random factor. To examine differences in cortisol levels, we log-transformed data (as cortisol data were not normally distributed) and an ANOVA was performed on differences between no-noise control animals and noise-exposed animals. The dependent variables in this experiment were behavioural markers (activity or sheltering response), cortisol levels, and ciliary bundle count. The fixed factor in the low-intensity treatment consisted of sound exposure (no-noise control or 140 dB re 1 μ Pa).

During the second high-intensity noise experiment (160 and 170 dB re 1 μ Pa), a one-way ANOVA was also used to examine behavioural differences of bullhead during baseline control and noise treatments. We used a Tukey post hoc test to further investigate where differences were present. Cortisol data were log-transformed and analyzed using an ANOVA, and ciliary bundle data were compared using an independent sample *t* test. The dependent variables in the high-intensity treatment were activity or sheltering, cortisol levels, and ciliary bundle counts; however, the fixed factor consisted of sound exposure (control or 160 or 170 dB re 1 μ Pa) and time of exposure (short- and long-period acute exposure).

Results

When exposed to 140 dB re 1 μ Pa boat noise, bullhead exhibited significant changes in behavioural characteristics. Activity levels decreased from 2.63 to 0.97 (±0.43 SE) minutes per hour when fish were exposed to boat noise played at 140 dB re 1 μ Pa ($F_{11.51}$ = 8.4,

p = 0.034; Fig. 5A). Sheltering behaviour increased from 21.37 to 24.27 (±1.21913 SE) minutes per hour when fish were exposed to the same noise ($F_{[1,5]} = 8.6$, p = 0.033; Fig. 5B). There was no significant difference in cortisol levels relative to resting levels ($F_{[8,50]} = 4.2$, p = 0.184; Fig. 5C) when exposed to 140 dB re 1 µPa for 4 h. When comparing ciliary bundle data in the control and noise treatment during the 140 dB re 1 µPa noise exposure, there was no significant difference in counts ($t_{[6]} = 0.78$, p = 0.902; Fig. 5D).

During the 160 and 170 dB re 1 µPa noise exposure treatments, differences were present within the short-period acute exposure experiment (1 h) for both activity and sheltering. Activity levels significantly decreased from 21.69 to 1.97 (±1.0 SE) minutes per hour during 160 dB re 1 µPa exposure and from 21.69 to 8.90 (±1.0 SE) minutes at 170 dB re 1 µPa ($F_{[2,12]}$ = 32.987, p < 0.001; Fig. 6A). Sheltering behaviour significantly increased from 27.13 to 57.70 (±0.80509 SE) minutes during 160 dB re 1 µPa and from 27.13 to 50.70 (±1.85 SE) minutes per hour during 170 dB re 1 µPa when bullhead were exposed to noise ($F_{[2,12]} = 11.236$, p < 0.001; Fig. 6B). During the long-period acute exposure (24 h), there were differences in both activity and sheltering behaviours. Overall, activity levels significantly decreased from 2.51 to 0 (±0.034 SE) minutes per hour at 160 dB re 1 µPa treatment and increased from 2.51 to 8.36 (±1.0 SE) minutes at 170 dB re 1 μ Pa ($F_{[2,12]}$ = 9.989, p = 0.002; Fig. 6A). Post hoc tests demonstrated significant differences in activity levels between the control treatment and 170 dB re 1 µPa of noise (p = 0.020) and between 160 and 170 dB re 1 μ Pa (p = 0.002), indicating that fish were more active during longer (24 h) exposure of Mickle et al.





Fig. 5. (A) Mean activity levels of black bullhead during exposure to 140 dB re 1 μ Pa of boat noise and during control trials. (B) Mean levels of sheltering behaviour in black bullhead when exposed to 140 dB re 1 μ Pa of boat noise and during control trials. (C) Mean log cortisol levels of black bullhead after exposure to 140 dB re 1 μ Pa of boat noise compared with controls. (D) Mean hair cell counts of black bullhead when exposed to 140 dB re 1 μ Pa of boat noise and in control trials. For all panels, significant differences are indicated by different letters at the *p* = 0.05 level. Error bars are representative of mean (±SE).



170 dB re 1 µPa compared with the control treatment. However, there was no significant difference in activity levels between the control and 160 dB re 1 μ Pa treatment (p = 0.442). Sheltering behaviour differed overall when bullhead were exposed to noise $(F_{[2,12]} = 10.799, p = 0.001;$ Fig. 6B). Post hoc tests indicated a nonsignificant difference in sheltering between the control treatment and 170 dB re 1 μ Pa (p = 0.072) of noise and also between control treatment and 160 dB re 1 μ Pa (p = 0.095). However, bullhead sheltered more at 160 dB re 1 μ Pa (60 min \cdot h⁻¹) compared with 170 dB re 1 μ Pa (50 min·h⁻¹) (p = 0.001). During acute exposure of both noise levels of 160 and 170 dB re 1 µPa, bullhead did not exhibit a change in cortisol levels compared with the control $(F_{[2,14]} = 1.305, p = 0.302; Fig. 6C)$. Cortisol data collected during the chronic noise treatment uncovered no significant differences $(F_{[2,15]} = 3.268, p = 0.066; Fig. 6C)$. Post hoc analyses revealed no significant difference between cortisol levels in the no-noise control experiment compared with 160 dB re 1 μ Pa (p = 0.147) and 170 dB re 1 μ Pa (p = 0.992) and between the no-noise control and 170 dB re 1 μ Pa (p = 0.075).

There was a significant effect of 160 and 170 dB re 1 μ Pa noise exposure on ciliary bundle number ($F_{[2,30]} = 18.458$, p < 0.001; Fig. 6D), resulting in fewer ciliary bundles present in noise treatments than no-noise controls. Post hoc tests further uncovered a

significant difference in ciliary bundles of hair cells when comparing the no-noise control and 170 dB re 1 µPa treatment (p < 0.001) during short-term acute exposure. During long-term exposure of noise, post hoc analyses determined a significant difference in ciliary bundle number between the control and 170 dB re 1 µPa (p < 0.001) and between 160 and 170 dB re 1 µPa (p = 0.001); however, there was not a significant difference between the no-noise control and 160 dB re 1 µPa (p = 0.062; Fig. 6D). There was no significant difference in the number of ciliary bundles present in each box placed along the saccular epithelium in both control and exposed ears (p = 0.0727), showing no regional effects of sound exposure on hair cell damage (Fig. 4).

Discussion

Anthropogenic noise caused a change in behavioural characteristics and ciliary bundles in black bullhead. Bullhead exhibited an increase in sheltering behaviour and a decrease in activity levels even when exposed to 140 dB re 1 μ Pa and had fewer ciliary bundles when exposed to 170 dB re 1 μ Pa during both short- and long-period acute exposure. Noise pollution research is not commonly studied in freshwater environments (Mickle and Higgs 2018), even though these environments are species-rich and im**Fig. 6.** (A) Mean activity levels of bullhead when exposed to 160 and 170 dB dB re 1 μ Pa of boat noise at two time points. (B) Mean sheltering levels of bullhead when exposed to 160 and 170 dB re 1 μ Pa of boat noise at two time points. (C) Mean cortisol levels of bullhead when exposed to 160 and 170 dB re 1 μ Pa of boat noise at two time points. (C) Mean cortisol levels of bullhead when exposed to 160 and 170 dB re 1 μ Pa of boat noise at two time points. (D) Mean hair cell counts of bullhead when exposed to 160 and 170 dB re 1 μ Pa of boat noise at two time points. For all panels, significant differences are indicated by different letters at the *p* = 0.05 level. Error bars are representative of mean (±SE).



portant economically and recreationally, as we rely on fish as a major source of protein for the world's population (16%) (Tidwell and Allan 2001). As such, we would suggest that more resources be dedicated to better understanding possible fitness effects of anthropogenic noise in these critical habitats.

Behavioural responses

The decreased activity patterns demonstrated by bullhead when exposed to 140 dB re 1 μ Pa suggest this level of anthropogenic noise impacts behavioural responses that can be a precursor to a physiological stress response (Eriksson and Van Veen 1980; Valdimarsson and Metcalfe 1998). Contrary to some literature (Smyly 1957; Lelek 1987), bullhead are not normally sedentary in nature, but instead are mobile (usually under dark conditions) to detect prey species and find suitable spawning habitats (Eriksson and Van Veen 1980; Knaepkens et al. 2004). Research has indicated that fish can often exhibit avoidance behaviours (Ona and Godø 1990; Fewtrell and McCauley 2012) in response to noise; however, because of constraints of tank size, we used activity levels to indicate a change in behavioural characteristics. McLaughlin and Kunc (2015) examined the behavioural impacts of boat noise on the convict cichlid (*Amatitlania nigrofasciata*) and found that although the presence of a boat noise increased time spent sheltering and decreased time spent foraging, it did not alter their overall activity level. Activity effects in bullhead may be due to the enhanced hearing capability of bullhead and suggests caution in extrapolating effects between species with different hearing abilities.

Physiological responses

Cortisol levels were used as a measure of physiological stress when fish were exposed to noise (Donaldson 1981; Wysocki et al. 2006; Barcellos et al. 2007). While our behavioural results suggested that fish were showing a stress response, this was not indicated by the physiological marker of cortisol level. This apparent discrepancy can be explained by the principle that animals first respond to stress through a behavioural mechanism (Dawkins 2003; Moberg and Mench 2005). It is also possible that there was no clear pattern associated with cortisol data due to sampling at the end of the experiment. If bullhead exhibited a spike in cortisol at the beginning of the experiment when fish were first introduced to the noise, it is possible cortisol levels returned to baseline levels after a certain amount of time had passed. Thus, our findings do not suggest that bullhead do not exhibit signs of physiological stress; to confirm this, more stress markers such as glucose, lactate, cardiac output, and changes in oxidative stress or immune response could be measured (Graham and Cooke 2008; Dantzer et al. 2014). Finally, physiological responses are highly context-specific and can be modified by a number of intrinsic and extrinsic factors (Madliger and Love 2014). As a result, physiological stress and changes in growth and condition may only be apparent after longer time periods or repeated exposures to noise stressors, and the extent of these responses may be different during different life history stages (Dantzer et al. 2014; Shannon et al. 2016).

Ciliary bundle data

Based on results obtained from the first noise treatment of 140 dB re 1 µPa, we decided to expose bullhead to both short- and long-term acute periods of 160 and 170 dB re 1 µPa to determine what the impacts were at these higher noise levels. As fish were being housed in communal tanks, we changed the experimental design during the higher intensity noise treatment to more accurately represent normal housing conditions and reduce stress in the chronic treatments that necessitated holding fish for longer time. Therefore, we had three fish in an experimental tank as opposed to one fish in six separate tanks. The boat noise played to the bullhead at these higher noise levels is still ecologically relevant — small boats generally produce noise ranging from 140 to 167 dB re 1 µPa, and merchant ships produce noise ranging from 178 to 192 dB re 1 µPa up to an approximate distance of 2 m away (Arveson and Vendittis 2000; Amoser et al. 2004). Cargo ships have been shown to reach noise levels up to 212 dB re 1 µPa at 1 m away; this level can create shock waves emitted from the propeller (Arveson and Vendittis 2000). During long-term acute exposure, fish were less active at 160 dB re 1 µPa but were more active during 170 dB re 1 µPa when compared with the control. These behavioural effects may be attributed to the ciliary bundle data; if there is a decrease in ciliary bundles of hair cells after both short- and long-term acute stressors of noise played at 170 dB re 1 µPa, bullhead hearing sensitivity will likely decrease (Smith et al. 2004), so they may no longer perceive the noise to be as loud and therefore stressful. If fish are no longer sensitive to the noise, it is likely they will no longer exhibit signs of stress, explaining why bullhead exposed to 170 dB re 1 μ Pa for 24 h did not exhibit a change in cortisol levels and were more active during this treatment, even relative to the controls that still had background noise present in the holding conditions.

Higgs and colleagues (2002) looked at regional differences in hair cell density along 14 regions of the saccular epithelium of zebrafish and only found density differences at the caudal end of the epithelium. Smith and colleagues (2003) counted hair cells along four locations (2500 μ m² boxes) along the saccular macula in goldfish; therefore, we focused on hair cell number along three locations of the bullhead saccule. We found no significant regional differences in both the controls and exposed ears. Previous research suggest topographic frequency-dependent loss of hair cells in fish (Furukawa and Ishii 1967); however, our boat noise file featured a broad spectral range (100–10 000 Hz), limiting the topographic effect of frequency specific hair cell damage.

Future considerations

There are a few considerations when analyzing the current data. First, some fish have higher baseline cortisol levels than others, which can cause variability in results. Second, cortisol levels fluctuate seasonally and diurnally (Laidley and Leatherland 1988); to avoid this confounding variable, all experiments were started at approximately the same time each day, over the period of 3 months. Owing to the capture of live fish, we had a large size range in bullhead; however, the fish were nonreproductive (fish were dissected to visualize presence of gonads) to avoid the impacts reproduction could have on behaviour. As we did not test other sources of noise, we cannot definitively say fish are respond-

ing to the boat noise specifically; however, we can conclude that bullhead display behavioural changes and fewer ciliary bundles when exposed to noise. As our research includes intensities of noise at 160 and 170 dB re 1 μ Pa, we need to determine the frequency of these noise levels in fishes' environment. Most research involving soundscape data are carried out in marine environments (McWilliam and Hawkins 2013; Staaterman et al. 2014; Erbe et al. 2015), but the data that do exist for fresh water (e.g., Amoser et al. 2004; Graham and Cooke 2008) do indicate that anthropogenic noise levels in freshwater ecosystems often exceed those used here.

Possible next steps for future study would be to measure sound levels in local areas across areas such as the Great Lakes impacted by boat noise to determine the source, timing, and duration of noise levels. Further research is also needed to determine boat noise impacts on freshwater fish with general hearing capabilities. Another component to consider is that the fish were kept in captivity and could not escape; therefore, in the wild fish may simply leave the area to avoid the noise. However, depending on the noise source, how loud it is, and health status of the animal, this may not always be possible. Recommendations to decrease noise impacts on freshwater habitats include the addition of protected areas, restricting human access to specific sites (particularly spawning grounds for endangered fish), the use of physical barriers to noise, and widespread quiet technology (Shannon et al. 2016). Noise pollution research is not commonly studied in freshwater environments (Mickle and Higgs 2018), even though these environments are species-rich and important for human survival (Tidwell and Allan 2001). More focus should be given to noise impacts on freshwater environments to be able to truly assess the impact of anthropogenic stressors on survival and fitness of these key species.

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