Fisheries Research 190 (2017) 1–14

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Effects of hydrokinetic turbine sound on the behavior of four species of fish within an experimental mesocosm

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ARTICLE INFO

Article history:
Received 15 June 2016
Received in revised form 20 January 2017
Accepted 24 January 2017
Prof. George A. Rose
Available online 4 February 2017

Keywords:
Hydrokinetic turbine
Underwater sound
Renewable energy
Fish behavior

ABSTRACT

The development of hydrokinetic energy technologies (e.g., tidal turbines) has raised concern over the potential impacts of underwater sound produced by hydrokinetic turbines on fish species likely to encounter these turbines. To assess the potential for behavioral impacts, we exposed four species of fish to varying intensities of recorded hydrokinetic turbine sound in a semi-natural environment. Although we tested freshwater species (redhorse suckers [*Moxostoma spp*], freshwater drum [*Aplondinotus grunniens*], largemouth bass [*Micropterus salmoides*], and rainbow trout [*Oncorhynchus mykiss*]), these species are also representative of the hearing physiology and sensitivity of estuarine species that would be affected at tidal energy sites. We evaluated changes in fish position relative to different intensities of turbine sound as well as trends in location over time with linear mixed-effects and generalized additive mixed models. We also evaluated changes in the proportion of near-source detections relative to sound intensity and exposure time with generalized linear mixed models and generalized additive models. Models indicated that redhorse suckers may respond to sustained turbine sound by increasing distance from the sound source. Freshwater drum models suggested a mixed response to turbine sound, and largemouth bass and rainbow trout models did not indicate any likely responses to turbine sound. Findings highlight the importance for future research to utilize accurate localization systems, different species, validated sound transmission distances, and to consider different types of behavioral responses to different turbine designs and to the cumulative sound of arrays of multiple turbines.

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1. Introduction

There has been increasing interest in the development of hydrokinetic (HK) energy technologies, in particular turbine based technologies that extract energy from free flowing and tidal currents in coastal and riverine environments (Čada et al., 2007). Researchers estimate that approximately 342–454 TWh/year of technically recoverable HK energy potential exists within US rivers and tidal zones, equivalent to 8–11% of 2012 US electrical consumption (Ravens et al., 2012; Haas et al., 2011). While HK turbine deployment has been limited, due in part to uncertainty surrounding environmental impacts (Čada et al., 2007), studies of environmental impacts at the individual turbine scale have evaluated the behavioral impacts of HK turbines and infrastructure on fish (Bevelhimer et al., 2013; Castro-Santos and Haro, 2015; Hammar et al., 2013; Viehman and Zydlewski, 2014), as well as the probability of HK turbine entrainment, blade strike, and injury (Amaral et al., 2015; Hammar et al., 2015). However, with only a few HK turbines currently deployed and a wide range of sizes and designs proposed, few studies have investigated the behavioral impact of HK turbine sound emissions on fish or other aquatic organisms.

Bevelhimer et al. (2016) characterized the sound levels produced by a typical tidal turbine relative to other anthropogenic sources of underwater sound and compared the sound intensity to fish hearing thresholds, but they did not conduct experiments with fish. Halvorsen et al. (2011) investigated the potential of injury and hearing damage to Chinook salmon (*Oncorhynchus tshawytscha)* exposed to simulated HK turbine sound with an average sound pressure level (SPL_{rms}) of 163 dB re 1 μPa for 24 h and reported a low risk of injury or effects on hearing. This likely represents a worst case exposure scenario with large diameter (>5 m) HK turbine sound emission levels estimated to range between 159 and 175 dB re 1 μPa at 1 m (Halvorsen et al., 2011; Hammar et al., 2015). In a comprehensive review of assessing the impact of underwater noise, Hawkins and Popper (2016) concluded that behavioral responses would likely occur at a greater distance from the sound

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http://dx.doi.org/10.1016/j.fishres.2017.01.012
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source than any other responses. Although sound of high enough amplitude to cause physical damage is possible and would be of great concern, sounds that are not damaging but that could affect normal behavior related to movements, feeding, and reproduction could still result in significant ecological consequences. For example, turbine avoidance could result in interrupted spawning migration and attraction (less likely but possible) toward a turbine could result in increased exposure to possible turbine blade strike.

The potential for HK sound to attract or repel fish is a particular concern in the context of turbines located near anadromous fish passage routes in rivers and estuaries or turbines located near preferred habitat and feeding areas of resident fish species. However, assessing these behavioral responses to sound in lab studies is challenging because of the difficulty in reliably replicating sound conditions in the lab due to reflection of sound within tanks (Akamatsu et al., 2002; Popper and Hastings, 2009). Furthermore, confinement might mask behavioral responses that would occur in the field (Popper and Hastings, 2009). Although field studies provide the most realistic exposure, the lack of such studies has hindered progress in identifying potential impacts (Hawkins and Popper 2016). On the other hand, in situ studies are limited by the lack of installed operational turbines where testing can take place. Due to these limitations, we elected to apply a mesocosm based approach to assess behavioral responses of four representative fish species to HK turbine sound.

To understand the likelihood of attraction or avoidance responses by fish to HK turbine sound and the distances at which responses might occur, we evaluated changes in fish location as a result of short-term (hour-long) and long-term (9–12 h) exposures to recorded turbine sound at multiple acoustic intensities in a semi-natural environment. Even though we are curious about any level of response to anthropogenic sources of sound, we are most interested in detecting behavioral responses that are large enough that they would likely have some ecologically relevant impact. Although one's first inclination is that unnatural sound would be a deterrent, perhaps because it is perceived as a predator or because it interferes with an organism's normal ability to sense predators we cannot rule out the possibility that turbine sounds could attract some fish. Therefore, we approached these studies with a mindset that either avoidance or attraction is possible and our methods and designed data analysis to detect either eventuality.

2. Methods

2.1. Study site and sound exposure system

The experiment was conducted in Kerr Hollow Quarry on the Oak Ridge Reservation, Anderson County, Tennessee. Restricted access to the site and its isolated location minimized the potential for interfering sounds. The quarry is approximately 1.2 ha in area with a maximum depth of approximately 15–20 m. Fish were contained in a floating net pen (19 × 6 × 1.5 m) constructed of 20-mm knotless nylon mesh netting oriented lengthwise near a north-south axis within the quarry. A net was placed over the entire pen to deter potential predators and deter escapement. Water temperature was monitored continuously during the experiments at the surface and at 1 m depth with data loggers (Model U22-001, Onset Computer Corporation, Bourne, MA, USA) deployed adjacent to the pen.

The sound exposure system consisted of an underwater speaker (Model AQ339, Clark Synthesis, Littleton, CO, USA) placed at each end of the pen at 1-m depth with each speaker oriented towards the middle of the pen. A two channel amplifier (Model PA660, Polk Audio, Baltimore, MD, USA) powered both speakers with separate portable media players (Model SDMX24, SanDisk Corporation, Milpitas, CA, USA) providing independent audio and volume control through each channel. Output from the sound exposure system was characterized with an underwater sound recording (USR) device (described in Martinez et al., 2011), hydrophones (Model Type 8104, Brüel & Kjær, DK-2850 Nærum, Denmark) calibrated with a Brüel & Kjær model 4229 pistonphone, and the Aquatic Acoustic Metrics Interface (AAMI) software package (version 1.2.2; Ren et al., 2012). The USR is a laboratory-tested, battery-powered recording device composed of a hydrophone signal processing board and digital data recorder capable of storing approximately 2 h of information from two hydrophones simultaneously. Sound recordings were taken at 11 locations throughout the pen, including directly in front of the speakers, in the corners, and the opposite end of the pen. We used the AAMI software to calculate peak SPL (SPL_{peak}) and root mean square SPL (SPL_{rms}), a common metric representing the average sound level, for 15–20 s of each recording.

We utilized a previously recorded HK turbine sound sample of Ocean Renewable Power Company's (ORPC, Portland, ME) TidGen® bottom-mounted horizontal axis turbine in Cobscook Bay, Maine. The sample was collected with a drifting calibrated hydrophone 21 m from the turbine and had a calculated SPL_{rms} of 121.2 dB re 1 uPa (Peter Stein, Scientific Solutions, Inc., personal communication). Bevelhimer et al. (2016) characterized and compared the turbine recording with anthropogenic sound sources using both spherical and cylindrical attenuation functions and estimated the sound amplitude SPL_{rms} at the turbine source at between 135 and 141 dB re 1 μPa. Based on this previous study, the SPL_{rms} recorded at each treatment level was regressed with the fitted $15\log_{10}(R)$ equation to provide an estimate of distances from a HK turbine that each treatment level might be encountered. We recognize that this is an estimate, but not having the ability to take additional measurements within a few meters of an operating HK turbine, we believe this was a reasonable way to choose experimental volumes and to provide an easily understandable interpretation of the volumes in terms of distance from a turbine.

The SPL_{rms} measured for each treatment level are presented in Fig. 1 (A&B). The mean ambient SPL_{rms} within the pen, represented by the control treatment, was 86.4 (±0.04 SE) dB re 1 μPa. The SPL_{rms} at 0.5 m from the speaker at high treatment volume measured 135.1 dB re 1 μPa, corresponding to a distance of 2.5 m from a turbine under practical spreading conditions. During the medium volume treatments, the SPL_{rms} at 0.5 m from the speaker measured 121.6 dB re 1 μPa, corresponding to a distance of 19.6 m from a turbine under practical spreading conditions. The SPL_{rms} of the low volume treatment measured 110.4 dB re 1 μPa at 0.5 m from the speaker, corresponding to a distance of 109.6 m from a turbine under practical spreading conditions. Because the original turbine recording was collected with a drifting hydrophone, there is some variation in sound amplitude within the looped playback of the 21-s original recording, including segments of a few seconds that are 1–2 dB higher than the 21-s SPL.

Although playback volumes were selected to produce SPLs that were representative of amplitudes around an operating turbine, the inexactness of sound reproduction by underwater speakers and the frequency-specific attenuation of sound in water produced sound signatures that were not identical to the original recording even though the overall amplitude was similar (Fig. 2). The realized frequency-specific sound amplitudes during these experiments was generally lower than the original recording at frequencies less than 200 Hz, but higher at frequencies up to 10,000 Hz for transmissions of similar overall amplitudes.

2.2. Ultrasonic tracking system

A time difference of arrival (TDOA) positioning system (Sonotronics, Tucson, AZ, USA) was used to track fish movements.

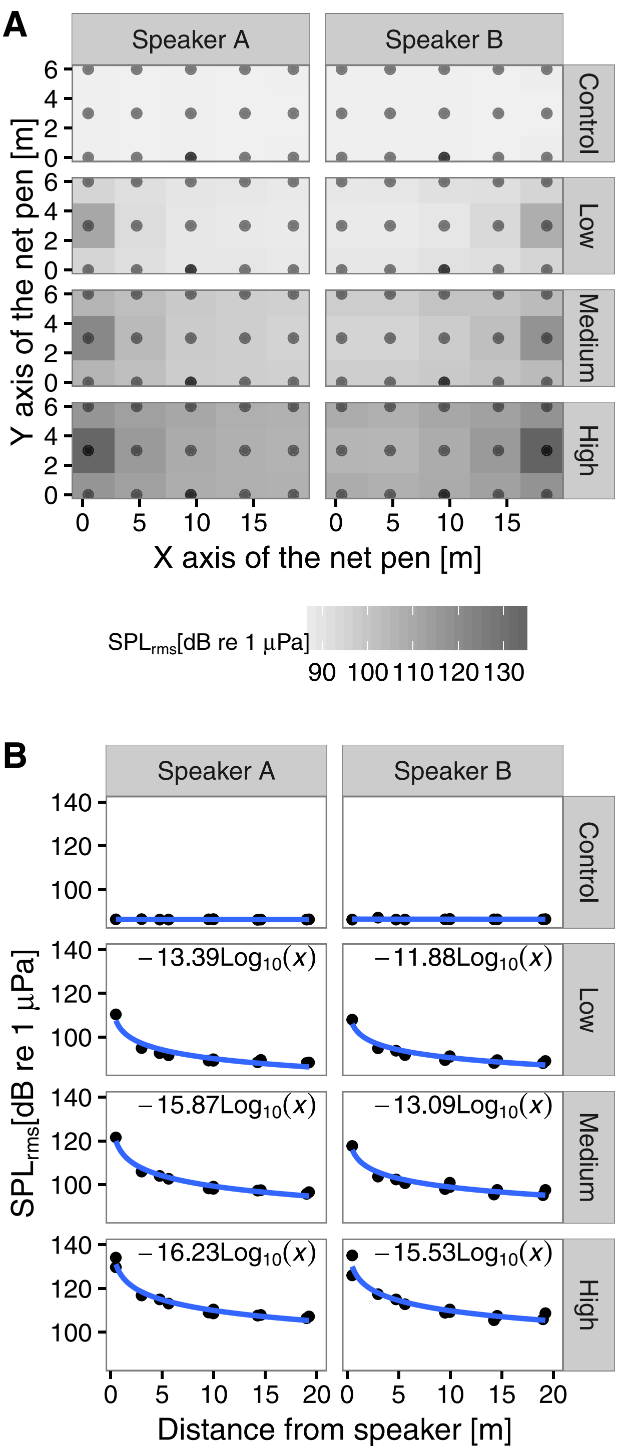

Fig. 1. (A) SPL_{rms} measured within the experimental pen at each volume level; points indicate locations where the SUR measured SPL_{rms}. Location measurements are assumed along the long (x) axis of the pen only. (B) Fitted transmission loss log functions for each volume x speaker combination.

The TDOA system consisted of a central unit that synchronized a timer, with accuracy of 0.625 ms, with four remote submersible ultrasonic receivers (SURs) connected via waterproof cables. The TDOA system detected and logged transmissions from implanted ultrasonic transmitters (IBT-92-1, Sonotronics) with a manufacturer estimated detection range of 100 m. With a manufacturer estimated error of ±0.625 milliseconds in the TDOA timer, the estimated error in localization was ±1.8m.

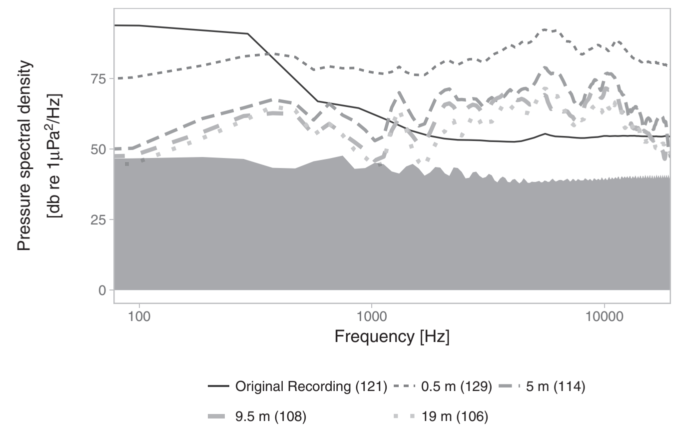

Fig. 2. Pressure spectral densities calculated from recordings at the high volume level taken at four distances from the underwater speaker compared to the original recording of the ORPC hydrokinetic turbine and ambient conditions in the quarry. Corresponding SPL_{rms} values for each recording are included parenthetically in the legend. Filled area indicates ambient pressure spectral density and assumed consistent across test conditions.

Tagged fish locations were determined by differences in the arrival time of each tag's unique signal at two or more receivers. Each transmitter emitted a signal at one of four frequencies (70, 72, 74, or 76 kHz) with unique transmission intervals that allowed for individual tag identification. One SUR was placed in each corner of the net pen at a depth of 1 m. The central unit also synchronized and changed the SUR detection frequencies every 27 s, allowing for each tag to be detected approximately once every 2 min under optimal conditions.

The resulting detection log files from each receiver were processed with the manufacturer's software to produce a single comma separated values (csv) file containing unique detections and associated timer data. A Python script identified detections and calculated a one dimensional location along the long axis of the pen based on the TDOA between pairs of receivers, speed of sound in water, and distance between receivers. Outliers, where the transmitter signal TDOA at two receivers translated to a distance greater than the physical distance between the two receivers, were removed from the analysis. For each species, 20–24% of control trial detections were removed and 24–27% of treatment trial detections were removed.

The positional accuracy of the TDOA system was evaluated in the field by deploying a test tag at known locations in the pen and calculating the root mean square error (RMS) between the locations calculated with TDOA system output and the known locations. The RMS error was calculated with a simplified procedure of an accuracy assessment used by Deng et al. (2011):

$$\Delta x_i = |x_{i\,solved} - x_{i\,actual}|, \quad i = 1, \ldots N$$

$$RMS = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\Delta x_i^2}$$

2.3. Fish species

Four fish species were chosen to evaluate attraction and avoidance behavior to HK turbine sound: redhorse suckers *Moxostoma* spp. (included black redhorse, *Moxostoma duquesni,* and silver redhorse, *M. anisurum*), freshwater drum (*Aplondinotus grunniens*), largemouth bass (*Micropterus salmoides*), and rainbow trout (*Oncorhynchus mykiss*) (Table 1). Although these are freshwater

Table 1
Fish and environmental condition summaries for each of four 2-wk trials.

Species	n	Test dates	Mean length	Mean weight	Mean water temperature
			(cm ± SE)	(g ± SE)	(C° ± SE)
Redhorse sucker	19	Sep 20–Sep 30	42.3 ± 1.0	824 ± 54	21.6 ± 0.04
Freshwater drum	12	Oct 06–Oct 16	49.6 ± 2.0	1879 ± 245	18.8 ± 0.02
Largemouth bass	14	Oct 27–Nov 07	37.5 ± 0.8	683 ± 45	14.7 ± 0.06
Rainbow trout	14	Nov 17–Nov 27	35.5 ± 0.4	406 ± 9	10.8 ± 0.02

species that were chosen as representative of species vulnerable to HK turbines in large river settings, they are also representative of the hearing physiology and sensitivity of many estuarine species that would be affected in tidal applications. Black and silver redhorse are part of the widely distributed family Catostomidae. Due to the presence of Weberian apparatus (an auditory structure connecting the swim bladder to the ear) in Catostomids, we anticipated that redhorse suckers likely have relatively high hearing sensitivity compared to species such as rainbow trout that lack morphological hearing specializations. Redhorse suckers are reasonable surrogates for a number of confamilial species of conservation interest, such as blue suckers (*Cycleptus elongates*), due to habitat and morphological similarities. They also represent other members of the Superorder Ostariophysi which is characterized by the presence of a Weberian apparatus and includes thousands of species worldwide (e.g., catfish and minnow species). Freshwater drum are a unique freshwater member of the family Sciaenidae, a family of fishes (including marine members) known for use of vocalization and extensive interspecific variations in sound detecting structures and abilities (Ramcharitar and Popper, 2004; Ramcharitar et al., 2006). Although unique, freshwater drum are a commonly encountered fish throughout the Mississippi River drainage which has high potential for HK development (Ravens et al., 2012). Largemouth bass are a member of the sunfish family Centrarchidae and lack hearing specialization structures, although a swim bladder is present relatively near the inner ear structure. Black bass (*Micropterus* spp.) have been widely introduced to rivers and reservoirs throughout the country and are likely to coincide with potential riverine HK developments. In addition, as the most popular sport fish in the US, with 443 million angling days spent on bass by 10.6 million anglers in 2011 (United States Department of the Interior and United States Department of Commerce, 2012), potential HK impacts on bass fisheries would likely generate public concern. Rainbow trout are another widely introduced fish lacking specialized hearing structures. We assumed that, due to similar morphology and habitat, rainbow trout serve as a convenient surrogate for congeneric trout and salmon species, including anadromous species of conservation, commercial, and recreational importance that would likely encounter HK turbines during migrations through rivers and estuaries.

Adult redhorse suckers and freshwater drum were collected via electrofishing in the Clinch River downstream of Melton Hill Dam (35.885372, −84.300195), and adult largemouth bass were collected by electrofishing upstream of Melton Hill Dam. Adult rainbow trout were obtained from the Tellico Trout Hatchery (Tennessee Wildlife Resources Agency, Tellico Plains, TN, USA). The number of fish used per trial was determined in part by the availability of healthy adult fish, the desire to maintain a reasonable density within the net pen, and limitations of the telemetry system. After accounting for occasional tag loss and net pen escape we analyze complete data for 19 redhorse suckers, 14 freshwater drum, 14 largemouth bass, and 14 rainbow trout. Mean fish length and weight are found in Table 1. Each species was tested in separate 2-wk trials to eliminate potential interspecific effects.

2.4. Fish tagging

Ultrasonic transmitters (22 × 9.5 mm and 2.5 g in water) were surgically implanted into the abdominal cavity of each fish. Fish were fasted for 12 h prior to anesthesia then placed in a 5 g/100 ml tricaine methanesulfonate (MS-222) buffered water bath solution until equilibrium was lost. Concentrations were increased as needed for some species which required a higher dose to lose equilibrium. Total length and weight were recorded and the fish placed in a surgical trough where water was circulated through the gills. A ventral incision was made and a transmitter implanted into the abdomen. The incision was closed with two or three sutures and the fish returned to a freshwater holding tank for visual observation until normal swimming resumed. Fish were monitored in holding tanks for one day to ensure return of normal swimming behavior before transfer to the outdoor net pen. Fish released into the net pen were acclimated for two days before exposure to recorded HK turbine sound.

2.5. Experimental design

The first portion of the experiment conducted during week 1 exposed fish to a series of two-hr treatments composed of alternating one hour sound exposure and one hour control (Fig. 3); these experiments will henceforth be referred to as the 'hourly exposure trials'. The exposure treatments consisted of continuous looped playing of the recorded turbine sounds through a randomly selected speaker. The control treatment consisted of a blank file played back through the audio system with all the hardware settings remaining the same as the exposure treatment. The control-treatment order was randomly chosen with the constraints that over the course of a trial the two speakers were used equally. Each group of fish were initially exposed to five pairs of exposure-control trials over one day at low SPL_{rms}, followed by 10 exposure-control trials over two days at medium SPL_{rms}, and a final 10 exposure-control trials over two days at high SPL_{rms}, for a total 25 pairs of exposure-control trials. Audio equipment malfunctions resulted in four missing redhorse sucker exposure-control trials (three at low volume and one at medium) and two missing freshwater drum exposure-control trials (at medium volumes).

The second portion of the experiment conducted during week 2 exposed fish to several 9- to 12-h periods of continual playback to determine if there was a significant trend in fish location during each treatment and whether long-term exposure might be interpreted as cumulative annoyance or habituation; these experiments will henceforth be referred to as 'long exposure trials'. Each trial exposed a group of fish to 9 h of HK turbine sound at medium SPL_{rms} from a randomly selected speaker, followed by 9 h at the opposite speaker. The group was then exposed to four different 9-h treatments of HK turbine sound at high SPL_{rms} from randomly ordered speakers (each speaker was used twice). Technical issues resulted in the redhorse suckers receiving four continuous treatments at 12 h in length, while all remaining species received six continuous

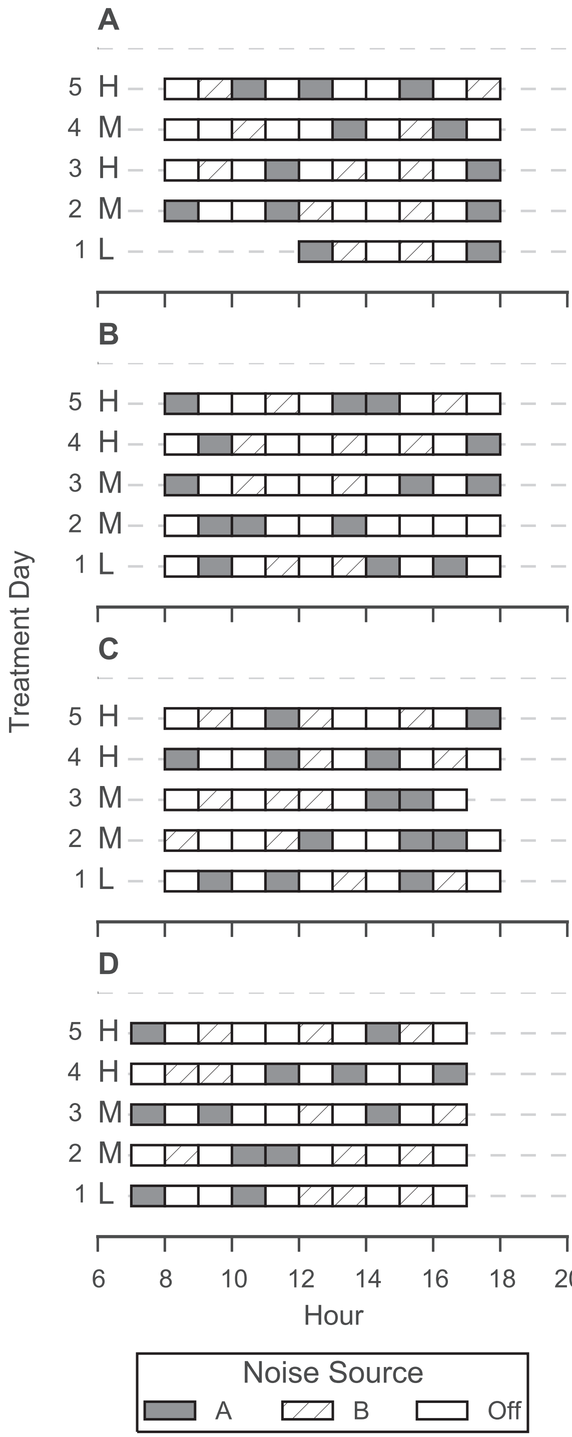

Fig. 3. Randomized exposure-control trials schedule for (A) redhorse sucker, (B) freshwater drum, (C) largemouth bass, and (D) rainbow trout. High (H), medium (M), and low (L) volume settings indicated along the left axis. Sound source indicated as solid colored box (speaker A), hatched box (speaker B), or hollow box (control).

treatments at 9 h in length with a 1 h break between treatments within a day and a 5 h break between days.

2.6. Data analysis

We evaluated behavioral response by comparing differences between exposure and control treatments using two location metrics: (1) mean distance from an active speaker and (2) proportion of time spent by a fish in close proximity (within 5 m) to the sound source. In addition, we tested in separate trials both short-term (week 1) and long-term (week 2) exposure and response in order to detect possible delayed response or acclimation. The two measures of behavioral response were evaluated via statistical methods chosen to test specific hypotheses – for week 1 trials a change in response to sound exposure relative to control treatments and for week 2 trials a trend in behavior relative to length of time of exposure. Embedded in these hypotheses was also a test of different magnitudes of sound exposure (i.e., playback volume).

2.6.1. Hourly exposure trials

Linear mixed effects models (LME) were used to analyze the effect of volume on fish position during the first week of treatment using the nlme package (Pineheiro et al., 2015) version 3.1–122 in R (R Development Core Team 2006). Separate models were fit for each species using all the detections collected during control and treatment periods; locations were not aggregated to mean locations. Experimental manipulation (speaker [S] and volume [V]), time (T, hours from the start of the experiment), and experimental day (D) were applied as fixed effects. Individual fish were included as a random effect to control for dependence due to repeated measures. The study is based on a planned comparison testing the effect of: (1) volume and (2) speaker. Therefore, the experimental manipulations were kept in all candidate models based on *a priori* expectations (Supplemental material Tables S1–S4) (Bolker et al., 2009; Anderson et al., 2001). Following Zuur et al. (2009), model selection started with a full model including each predictor variable and the two way interaction between experimental manipulations fitted with restricted maximum likelihood (REML) parameter estimation (three and four way interactions that included each predictor variable and experimental manipulation were left out due to the lack of model convergence). The full model was of form:

$$\text{Distance}_{i,j} = \beta_0 + \beta_1 D + \beta_2 T + \beta_3 V + \beta_4 S + \beta_5 V \times S + \alpha_i + \varepsilon_{i,j}$$

where $Distance_{i,j}$ is the distance of individual fish i, during observation j from the active sound source. β's are the coefficients of the fixed effects. α_i is the random intercept that allows variation between individuals and is normally distributed with mean zero. $\varepsilon_{i,j}$ is the residual term that represents within subject variation and is normally distributed with mean 0 and variance σ^2.

Different variance structures were compared and selected based on Akaike's Information Criterion (AIC) values. Tested variance structures allowed for a different variance per model variable. Temporal autocorrelation structures were also compared and selected based on the lowest AIC value. After random effects, variance, and correlation structures were finalized, each candidate model was fit using maximum likelihood (ML) parameter estimation and the final model chosen based on AIC (Zuur et al., 2009). Models were visually validated for normality and homogeneity by plotting normalized residuals against model variables and fitted values. Temporal autocorrelation was visually inspected with a semivariogram due to irregularly spaced detection data (Zuur et al., 2009). REML fitted models were used for final parameter estimation.

The sound field of the net pen indicated a SPL drop off in the first 5–10 m from each speaker, with generally equal SPL throughout the rest of the net pen (Fig. 1B). Therefore, a second analysis was conducted modeling the proportion of detections within 5 m of the active speaker during each treatment using generalized linear mixed models (GLMM) with a binomial distribution and logistic link function. Likelihood was approximated using Laplace approximation. Following model protocol used for LME's, separate models were fit for each species. Day (D), hour of day (H), volume (V), speaker (S), and an interaction term between speaker and volume were included in the full model. Experimental manipulations (V and S) were kept in all candidate models. Final models were chosen by

AIC and model assumptions visually assessed by plotting binned residuals (Gelman and Hill, 2007). The full GLMM was of form:

$$logit\left(\pi_{ij}\right) = n_{ij} = \beta_0 + \beta_1 D + \beta_2 H + \beta_3 V + \beta_4 S + \beta_5 S \times V + t_i + \varepsilon_{ij}$$

The residual term t_i accounts for correlation within observations of the same fish (t_i) and the additional residual term ε_{ij} is the observation level random intercept that is normally distributed with mean zero. The optimal model was selected based on AIC values. The lme4 package version1.1-12 (Bates et al., 2015) in R was used to fit GLMMs.

2.6.2. Long exposure trials

We used a generalized additive mixed model (GAMM) in mgcv (Wood, 2011) version 1.8-12 in R to model nonlinear trends in fish position over the course of each treatment volume. Due to restrictions on the data volume that could be handled by the GAMM, the data were subsampled to mean hourly fish locations and separate models were fit for each species. Following model selection protocol used with LME models, candidate models were selected *a priori* and included experimental manipulations in each model (Supplemental material Tables S5–S8). To allow for fluctuations based on volume, a volume specific smoother term for 'time from start of treatment' (L) was included. An hour (H) of day term was included and smoothed with a cyclic spline function to account for the assumption that fish behavior would be more similar at certain times of the day. Speaker (S) and volume (V) were also included in candidate models. Individual fish nested within treatment were included as a random effect to control for dependence due to repeated measures. Following Zuur et al. (2009) model selection began with a full candidate model fit with REML:

$$Distance_{i,j} = f(L) + f(H) + \beta_3 V + \beta_4 S + \beta_5 S \times S + \alpha_i + \varepsilon_{i,j}$$

where $f(L)$ is the volume specific smoothing function for the length of exposure, while $f(H)$ is the smoothing term for hour of day. Inclusion of variance and temporal autocorrelation structures followed the methodology used with LME models. After variance and correlation structures were finalized, candidate models were fit using ML estimation and selected using AIC values. Final models were fit with REML and model residuals visually assessed for normality and homogeneity. Temporal autocorrelation was visually assessed with the autocorrelation function.

We also modeled the proportion of hourly detections within 5 m of the tested speaker using a GAMM with a binomial distribution and logistic link function with the gamm4 (Wood and Scheipl, 2014) package version 0.2-3 in R. Likelihood was approximated with Laplace approximation. The fixed and smoothed model terms of candidate models were the same used in GAMM distance models. The full model was of form:

$$logit\left(\pi_{ij}\right) = n_{ij} = f(L) + f(H) + \beta_2 V + \beta_3 V + \beta_4 S + \beta_5 V \times S + \alpha_i + \varepsilon_{ij}$$

Software limitations prevented the inclusion of temporal autocorrelation structures. Candidate models were compared and selected with AIC. The selected model was visually assessed for normality and heterogeneity.

3. Results

3.1. TDOA system effectiveness

Although TDOA acoustic localization systems have been used with high accuracy in fine-scale 2D and 3D localization (Biesinger et al., 2013; Deng et al., 2011) our preliminary validation attempts at 2D localization were limited by the resolution of the time of arrival data collection, therefore we provide results with fish locations only along the long axis of the pen. The preliminary field test to evaluate system accuracy included 53 detections of an unimplanted acoustic tag with a RMS error of 2.06 m along the x-axis of the pen indicating reasonable accuracy of the TDOA system (Fig. 4). During the trials with fish, the mean detections per hour for tag-implanted rainbow trout was 6.3 (±0.06 SE), for largemouth bass 6.8 (±0.05), for freshwater drum 10.4 (±0.11), and for redhorse sucker 7.4 (±0.07).

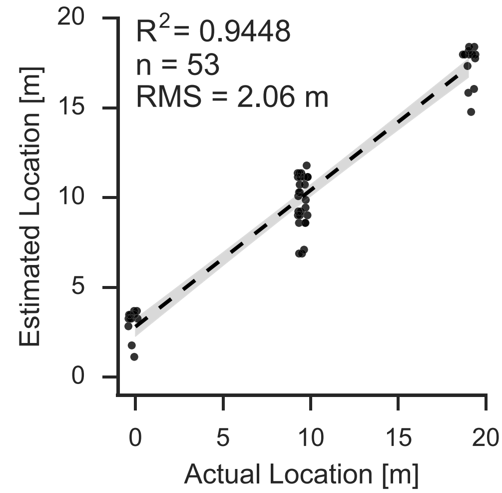

Fig. 4. Linear regression between known tag locations and locations estimated with the TDOA system, shaded area is the 95% confidence interval.

3.2. Hourly exposure trials

3.2.1. Redhorse suckers

The selected LME model for redhorse suckers included day, volume, and speaker (Supplemental Table S1). The selected redhorse sucker model indicated that low volume had a small weakly negative effect on redhorse sucker distance (Table 2, Fig. 5A). The LME model indicated that other volumes and speaker source did not have a significant effect on redhorse sucker distance. The selected redhorse GLMM for the proportion of detections within 5 m of the sound source included day, hour, volume, and the volume:speaker interaction (Supplemental Table S5). The GLMM indicated a significant decrease in redhorse sucker, near-source detections at high volume ($z = -4.39$, $p < 0.001$, Table 3, Fig. 5B) and a weakly significant speaker effect ($z = 1.99$, $p = 0.046$).

3.2.2. Freshwater drum

The selected LME model for freshwater drum location included time, day, volume, speaker, and the speaker, volume interaction term (Supplemental Table S2). Both speaker and volume had significant effects on freshwater drum distance (Table 2). When sound emanated from speaker A, the drum moved closer to the speaker at high volumes (Fig. 5A). However, when speaker B was the sound source, freshwater drum moved further away at low and medium volumes. A difference was not detected between no sound and high volume during speaker B treatments. The selected freshwater drum GLMM for the proportion of detections within 5 m included hour, volume, speaker, and the speaker, volume interaction (Table S6). The model indicated no significant effect at different volumes (Table 3, Fig. 5B)

3.2.3. Largemouth bass

The selected LME model for largemouth bass location included time, day, volume, and speaker (Supplemental material Table S3). High volume had a small significant positive effect on largemouth bass distance, while low and medium did not have a significant

Table 2
REML parameter estimates from the best LME model for each species, relating distance from speaker to fixed effects during hourly exposure experiments.

Parameter	Lower CI	Estimate	Upper CI	t-value	p
Redhorse suckers					
Intercept	8.24	8.99	9.75	23.31	<0.001
Day (2)	−0.29	0.57	1.42	1.30	0.193
Day (3)	0.55	1.35	2.16	3.30	0.001
Day (4)	−0.70	0.17	1.05	0.39	0.696
Day (5)	0.30	1.13	1.96	2.66	0.008
Volume (Low)	−1.55	−0.78	−0.01	−1.99	0.046
Volume (Medium)	−0.16	0.30	0.77	1.29	0.198
Volume (High)	−0.25	0.12	0.49	0.63	0.530
Speaker (B)	−0.03	0.30	0.63	1.76	0.079
Freshwater drum					
Intercept	12.21	13.40	14.14	26.51	<0.001
Time	−0.24	−0.18	−0.07	−4.19	<0.001
Day (2)	3.16	5.70	7.47	5.18	<0.001
Day (3)	1.91	7.06	10.12	3.36	<0.001
Day (4)	7.95	13.92	19.97	4.52	<0.001
Day (5)	8.89	17.57	24.78	4.31	<0.001
Volume (Low)	1.56	2.42	3.43	4.68	<0.001
Volume (Medium)	−0.38	0.23	0.91	0.68	0.495
Volume (High)	−3.23	−1.60	−2.25	−6.20	<0.001
Speaker (B)	−7.76	−6.98	−6.72	−25.77	<0.001
Volume (Low): Speaker (B)	1.37	2.80	4.21	4.05	<0.001
Volume (Medium): Speaker (B)	5.11	5.92	7.34	10.21	<0.001
Volume (High): Speaker (B)	2.59	2.88	3.92	8.25	<0.001
Largemouth bass					
Intercept	10.30	11.19	12.08	24.55	<0.001
Time	−0.14	−0.06	0.02	−1.50	0.135
Day (2)	−1.69	0.43	2.54	0.39	0.693
Day (3)	−0.45	3.50	7.45	1.74	0.083
Day (4)	−1.30	4.49	10.28	1.52	0.128
Day (5)	−3.40	4.30	11.99	1.10	0.273
Volume (Low)	−0.98	−0.09	0.79	−0.21	0.834
Volume (Medium)	−0.59	−0.06	0.47	−0.22	0.826
Volume (High)	0.10	0.57	1.04	2.37	0.018
Speaker (B)	−1.15	−0.73	−0.31	−3.42	<0.001
Rainbow trout					
Intercept	7.66	8.34	9.01	24.28	<0.001
Day (2)	1.82	2.84	3.86	5.46	<0.001
Day (3)	1.10	2.10	3.11	4.10	<0.001
Day (4)	3.05	3.95	4.86	8.55	<0.001
Day (5)	1.15	2.08	3.00	4.41	<0.001
Volume (Low)	−0.13	0.48	1.10	1.54	0.124
Volume (Medium)	0.02	0.50	0.98	2.05	0.040
Volume (High)	−0.36	0.06	0.48	0.27	0.785
Speaker (B)	−2.84	−2.41	−1.98	−11.01	<0.001

effect (Table 2, Fig. 5A). We also observed a small significant effect due to speaker. The selected largemouth bass GLMM model for the proportion of detections within 5 m included day, volume, and speaker fixed terms (Supplemental Table S7). The model indicated a significant speaker effect ($z = 3.07$, $p = 0.002$) but no effects from volume (Table 3, Fig. 5B).

3.2.4. Rainbow trout

The selected rainbow trout location LME model included day, volume, and speaker (Supplemental material Table S4). Low and high volume conditions did not have an effect on rainbow trout distance (Table 2, Fig. 5A). Medium volume had a small weakly significant effect on rainbow trout distance. A larger effect was observed between speakers. The GLMM model for the proportion of rainbow trout detections within 5 m included hour, volume, speaker, and the volume, speaker interaction (Supplemental Table S8). The model indicated a weakly significant decrease in detections at low volumes ($z = -2.12$, $p = 0.034$, Table 3) and a significant decrease in detections at high volume ($z = -2.72$, $p = 0.006$, Table 3, Fig. 5B).

3.3. Long exposure trials

3.3.1. Redhorse suckers

GAMM model selection relating redhorse sucker distance to fixed effects resulted in a model that included volume, speaker, their interaction, a volume specific smoothing term for exposure length, and a smoother term for hour of day (Supplemental material Table S9). Parameter estimates are provided in Table 4. At medium and high volumes, redhorse suckers moved away from the speaker as exposure length increased (Fig. 6A). The trend is immediate at high volumes while the trend is delayed at medium volumes. The selected GAMM redhorse model for the proportion of redhorse sucker detections within 5 m included volume, speaker, their interaction, a volume specific exposure length smoothing term, and smoothing term for hour of day (Supplemental material Table S13). Parameter estimates indicate near speaker detections decreased at high volumes ($z = -9.70$, $p < 0.001$, Table 5). The volume specific smoothing term indicated redhorse sucker near speaker detections linearly increased over exposure length at medium volumes (Fig. 6B). At high volumes, the smoother indicates an initial decrease

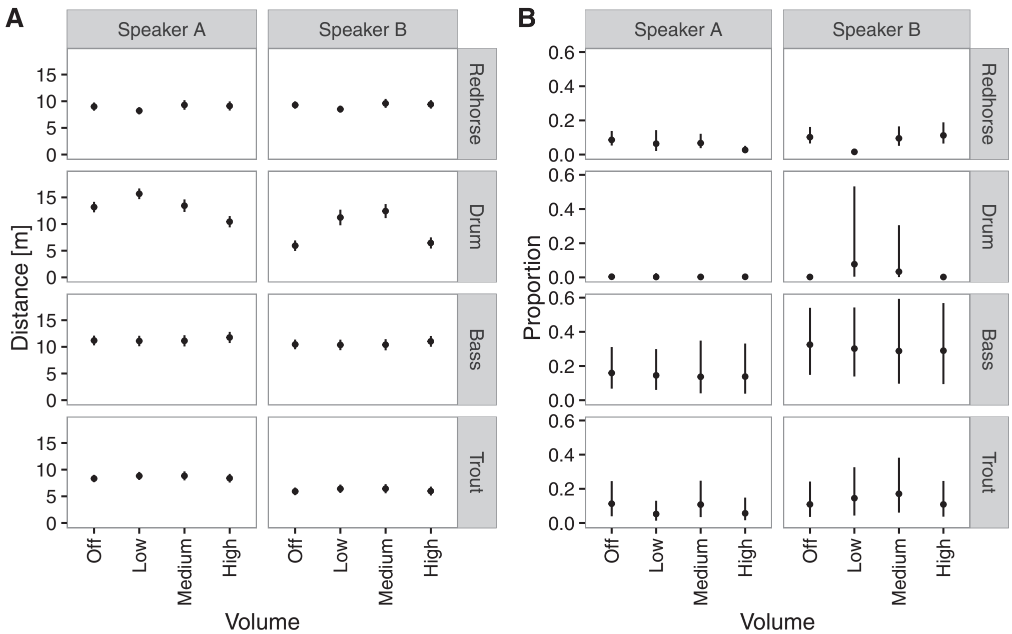

Fig. 5. Modeled (A) distances from speakers based on the best LME model and (B) proportions of detections within 5 m based on the best GLMM model for each species. Vertical lines indicate 95% confidence intervals.

in redhorse sucker near speaker detections that increases near the end of the exposure trial (Fig. 6B).

3.3.2. *Freshwater drum*

With freshwater drum, the selected GAMM model included volume, speaker, the interaction, a volume specific smoothing term for exposure length, and a smoother term for hour of day (Supplemental material Table S10). At high volumes, freshwater drum did not exhibit fluctuation in distance from the speaker (Fig. 6A). At medium volumes, freshwater drum exhibited initial movement away, followed by movement back towards the speaker after 2.5 h. The selected freshwater drum GAMM for the proportion of near speaker detections included volume, speaker, the interaction, the volume specific smoother for exposure length, and the smoothed hour of day terms (Supplemental material Table S14). The fixed effects parameter estimates did not indicate a significant difference in detections between volumes ($z = -1.74$, $p > 0.05$, Table 5). The volume specific exposure period smoother indicated that the proportion of near speaker detections remained near zero over the length of all exposures, except for medium volume from speaker B (Fig. 6B).

3.3.3. *Largemouth bass*

The selected GAMM model for largemouth bass location included exposure length, volume, speaker, the volume, speaker interaction, and a smoother term for hour of day (Supplemental material Table S11). The linear trend indicated that largemouth bass distance did not fluctuate over exposure length (Fig. 6A). The selected GAMM model relating the proportion of largemouth bass detections within 5 m to fixed effects included volume, speaker, the interaction, the volume specific exposure length smoother, and the hour of day smoother (Supplemental Table S15). The model indicated a significant increase in detections during high volume ($z = 7.36$, $p < 0.001$, Table 5). The proportion of near speaker detections decreased over each exposure period at both volumes (Fig. 6B).

3.3.4. *Rainbow trout*

GAMM model selection for rainbow trout location resulted in a model that included only volume, speaker, a volume specific smoothing term for exposure length, and a smoother term for hour of day (Supplemental material Table S12). A linear trend was present at high volumes with minor increase in distance over exposure length (Fig. 6A). A non-linear trend at medium volumes indicated minor fluctuation over exposure length, but changes in distance were small. The rainbow trout GAMM for the proportion of near speaker detections included volume, speaker, exposure length, and smoothed hour of day terms (Supplemental Table S16). There was a weakly significant effect between volumes ($z = -2.01$, $p = 0.044$, Table 5). The effect of exposure length on the proportion of rainbow trout near speaker detections was not significant ($z = -1.41$, $p = 0.160$, Table 5, Fig. 6B).

4. Discussion

The results of this study were mixed with some indication of avoidance response to the broadcast turbine sounds; however, there was not overwhelming evidence that a significant behavioral change should be expected in response to either short- or long-term exposure to HK turbine sound. The distance-from-speaker based models suggest that redhorse suckers did not demonstrate appreciable changes in mean location at the tested volumes. However, the proportion of near speaker redhorse sucker detections significantly decreased during short-term high volume treatments compared to controls. The proportion of near speaker redhorse sucker detection decreased over time during long-term exposures of high volume treatments, but showed a slight increase near the end of the treatment, suggesting some initial avoidance followed by possible acclimation toward the end of the long exposure. The distance based models for freshwater drum showed mixed results, and differences between speakers suggest other factors may have influenced drum position during the experiment. There were no significant differences in the proportion of near speaker freshwater drum detections at different volumes. The proportion of near speaker freshwater drum detections during the long exposure tri-

Table 3
Parameter estimates of fixed effects from the selected GLMM model of each species indicating the proportion of detections within 5 m of the source speaker during hourly exposure experiments.

Parameter	Estimate	SE	z-value	p
Redhorse suckers				
Intercept	−3.61	0.45	−8.07	<0.001
Hour	0.08	0.02	3.35	<0.001
Day (2)	1.25	0.37	3.26	0.001
Day (3)	1.19	0.37	2.81	0.005
Day (4)	1.05	0.37	2.89	0.004
Day (5)	1.08	0.37	3.22	0.001
Volume (Low)	0.74	0.55	1.34	0.180
Volume (Medium)	−0.28	0.25	−1.14	0.252
Volume (High)	−1.20	0.27	−4.39	<0.001
Speaker (B)	0.37	0.19	1.99	0.046
Volume (Low):Speaker(B)	−1.74	0.63	−2.75	0.006
Volume (Medium):Speaker(B)	0.01	0.33	0.03	0.980
Volume (High):Speaker(B)	1.12	0.34	3.32	<0.001
Freshwater drum				
Intercept	−5.57	0.79	−7.03	<0.001
Hour	0.38	0.10	3.76	<0.001
Volume (Low)	−0.42	1.18	−0.36	0.718
Volume (Medium)	−0.42	0.93	−0.46	0.647
Volume (High)	−0.10	0.99	−0.11	0.916
Speaker (B)	−0.57	0.80	−0.72	0.475
Volume (Low):Speaker(B)	4.07	1.80	2.27	0.023
Volume (Medium):Speaker(B)	3.18	1.66	1.92	0.055
Volume (High):Speaker(B)	0.07	1.43	0.05	0.964
Largemouth bass				
Intercept	−1.67	0.51	−3.30	<0.001
Day (2)	−1.09	0.60	−1.82	0.068
Day (3)	−0.65	0.62	−1.05	0.296
Day (4)	−1.22	0.60	−2.07	0.039
Day (5)	−1.75	0.60	−2.94	0.003
Volume (Low)	−0.11	0.62	−0.17	0.863
Volume (Medium)	−0.18	0.47	−0.37	0.712
Volume (High)	−0.17	0.46	−0.36	0.720
Speaker (B)	0.93	0.30	3.07	0.002
Rainbow trout				
Intercept	−2.06	0.50	−4.09	<0.001
Hour	−0.07	0.02	−2.72	0.007
Volume (Low)	−0.83	0.39	−2.12	0.034
Volume (Medium)	−0.05	0.24	−0.22	0.829
Volume (High)	−0.75	0.27	−2.72	0.006
Speaker (B)	−0.04	0.18	−0.21	0.832
Volume (Low):Speaker (B)	1.16	0.49	2.34	0.019
Volume (Medium):Speaker (B)	0.57	0.34	1.70	0.089
Volume (High):Speaker (B)	0.75	0.36	2.05	0.040

als slightly decreased over time, but the proportion of near speaker detections generally remained extremely low for all the freshwater drum trials. The largemouth bass models did not indicate differences in mean location at tested volumes, and the proportion of near speaker detections remained the same among volumes. However, the proportion of near speaker largemouth bass detections gradually decreased over time suggesting the possibility of slight avoidance behavior. The distance based rainbow trout models suggest trout locations were not impacted by volume. Interpretation of the distance based rainbow trout models is limited because distances were generally outside of 5 m. The proportion of near speaker rainbow trout detections decreased at high volumes, but remained stable over time.

Fish species are often classified as either hearing generalists or specialists based on the presence of morphological hearing specializations and ranges of hearing sensitivity (Popper, 2003). However, most fish species likely fall along a continuum of pressure and particle motion sensitivity ratios with some species only sensitive to particle acceleration at one end of the spectrum, other species mostly sensitive to acoustic pressure at the other end of the spectrum, and most fish falling somewhere in between (Fay and Popper, 2012; Popper and Fay, 2011). Ladich and Fay (2013) recently published a review of fish hearing studies that included audiograms for over 100 fish species. To gain a better understanding of hearing sensitivity of different fish species to sound produced by HK turbines, we compared the SPL of the ORPC turbine recording as played in the sound exposure system to published hearing thresholds of similar and related fish species to those tested in this study (Ren et al., 2012; Wysocki et al., 2007; Amoser and Ladich, 2005; Mann et al., 2007; Fig. 7). Data were available for rainbow trout, but not specifically for the other species. Largemouth bass are represented by redeye bass (*Micropterus coosae*), a congeneric species; redhorse sucker are represented by longnose sucker (*Catostomus catostomus*), a member of the same subfamily, Catostominae; and freshwater drum are represented by black drum (*Pogonias cromis*), a marine species within the same family Sciaenidae. For comparison, the audiogram for common carp (*Cyprinus carpio*) was included as a representative of species with a hearing threshold lower than most, American shad (*Alosa sapidissoma*) as a representative of a species with a very broad hearing spectrum, and Chinook salmon because they represent a family of fish (Salmonidae) with world-wide commercial importance and are a congeneric of rainbow trout. The frequency

Table 4
Parameter estimates and approximate significance of smooth terms from the best GAMM model for each species relating distance from transmitting speaker to fixed effects during long-term exposure experiments.

Parametric terms	*Lower CI*	*Estimate*	*Upper CI*	*t-value*	*p*
Redhorse suckers					
Intercept	8.28	9.66	11.04	13.77	<0.001
Volume (High)	0.26	1.94	3.63	2.31	0.021
Speaker (B)	−3.31	−1.20	0.91	−1.14	0.254
Volume (High): Speaker (B)	−6.27	−3.91	−1.55	−3.32	<0.001
Smooth terms		*edf* [a]		*F-value*	*p*
s(Exposure Length): Volume (Medium)		4.77		3.93	0.003
s(Exposure Length): Volume (High)		3.30		7.54	0.001
s(Hour of day)		12.34		4.11	<0.001
	Freshwater drum				
Intercept	13.48	14.33	15.18	48.60	<0.001
Volume (High)	0.50	1.44	2.37	4.89	<0.001
Speaker (B)	−5.82	−4.57	−3.32	−7.50	<0.001
Volume (High): Speaker (B)	−9.03	−7.70	−6.36	−12.53	<0.001
Smooth terms		*edf*		*F-value*	*p*
s(Exposure Length): Volume (Medium)		7.47		14.41	<0.001
s(Exposure Length): Volume (High)		7.90		3.25	0.002
s(Hour of day)		3.19		2.77	<0.001
Largemouth bass					
Intercept	9.11	10.56	12.01	14.32	<0.001
Exposure Length	−0.10	0.05	0.20	0.68	0.497
Volume (High)	−3.18	−1.68	−0.18	−2.24	0.025
Speaker (B)	−3.44	−1.59	0.25	−1.73	0.084
Volume (High): Speaker (B)	0.20	2.37	4.53	2.18	0.030
Smooth terms		*edf*		*F-value*	*p*
s(Hour of day)		9.7		1.9	<0.001
Rainbow trout					
Intercept	3.88	8.45	13.01	3.64	<0.001
Volume (High)	−1.36	3.31	7.99	1.42	0.155
Speaker (B)	−5.56	−4.39	−3.21	−7.50	<0.001
Smooth terms		*edf*		*F-value*	*p*
s(Exposure Length): Volume (Medium)		4.67		5.81	0.002
s(Exposure Length): Volume (High)		1.47		0.51	0.394
s(Hour of day)		3.66		0.90	<0.001

[a] Estimated degrees of freedom.

spectrum produced by broadcasting the turbine recording during the study exceeded the threshold of common carp and longnose sucker. The thresholds of redeye bass, Chinook salmon, and black drum were within a few dB of the turbine frequency spectrum. Note that these reported thresholds are mean values derived from laboratories studies that often included individual values several dB below the mean. Although we would expect related species to have similar hearing abilities, extrapolating results from one species to another should be done with caution (Popper and Hastings, 2009; Popper, 2003).

Mixed results and large confidence intervals in the freshwater drum models complicate any conclusions about this species' response to the turbine sound. The large size (~2 cm in diameter) of the sagital otoliths removed from the test drums suggests that this species is sensitive to sound pressure like its marine cousins, but, due to the significant variation in sound detection amongst sciaenids, applying these model results to other species of sciaenids should be considered carefully (Ramcharitar and Popper, 2004; Ramcharitar et al., 2006).With numerous sciaenid species associated with coastal and estuarine habitats, future attention should be given to the potential for HK sound to not only attract or repel sciaenids, but also to potential impacts of auditory masking which may impact predator/prey interactions and social or reproductive behaviors. Increases in ambient background noise have been reported to result in nearly linear increases in the hearing thresholds of other hearing specialists such as common carp (*Cyprinus carpio*), goldfish (*Carassius auratus*), and Raphael catfish (*Platydoras costatus*) (Amoser and Ladich, 2005; Wysocki and Ladich 2005). The interspecific differences observed among sciaenids in hearing thresholds and masked hearing thresholds (the shifted hearing threshold in response to a masking acoustic signal) (Ramcharitar and Popper, 2004) highlight the importance for future research to further assess the potential for species-specific behavioral impacts under a variety of acoustic exposure and background sound conditions.

In comparison to sciaenids, the current literature in the evaluation and description of the hearing capabilities for both catostomids and centrarchids is not as well described. Minimum hearing thresholds for many cyprinids, which possess a Weberian apparatus, indicate cyprinids have high hearing sensitivities (Amoser and Ladich, 2005; Wysocki and Ladich, 2005) which led to the expectation that the catostomid redhorse sucker, which also contains a Weberian apparatus, might exhibit a response to turbine sound. The GAMM provide evidence that redhorse sucker demonstrate some type of avoidance behavior over time, but may also acclimate to sound at the volumes tested. Without published hearing threshold data or audiograms for catostomids, we can only speculate at which intensities are required to initiate a response in redhorse suckers. By comparison, centrarchids, lacking hearing specializations, have demonstrated relatively low hearing sensitivities (Wysocki and Ladich, 2005). Holt and Johnston (2011) used an auditory brainstem response approach to determine a maximum hearing threshold of 86 dB and 90 dB at 100 Hz for congeneric redeye bass (*Micropterus coosae*) and Alabama bass (*Micropterus henshalli*) respectively (Fig. 7). The linear response at low frequencies found by Holt and Johnston (2011) and lack of structures connecting the swim bladder to the inner ear suggest that *M. coosae, M. henshalli*, and presumably *M. salmoides* are more sensitive to par-

Table 5
Parameter estimates and approximate significance of smooth terms from the selected GAMM model of each species indicating the proportion of detections within 5 m of the source speaker during long-term exposure experiments.

Parametric terms	*Estimate*	*SE*	*z-value*	*p*
Redhorse suckers				
Intercept	−1.18	0.27	−4.41	<0.001
Volume (High)	−1.22	0.13	−9.70	<0.001
Speaker (B)	−1.11	0.23	−4.79	<0.001
Volume (High): Speaker (B)	0.28	0.16	1.70	0.088
Smooth terms	*edf* [a]		*F-value*	*p*
s(Exposure Length): Volume (Medium)	1.00		14.46	<0.001
s(Exposure Length): Volume (High)	7.62		112.23	<0.001
s(Hour of day)	9.52		78.99	0.088
Freshwater drum				
Intercept	−3.33	0.74	−4.49	<0.001
Volume (High)	−1.57	0.90	−1.74	0.081
Speaker (B)	2.75	1.08	2.54	0.011
Volume (High): Speaker (B)	−2.59	1.31	−1.98	0.048
Smooth terms	*edf*		*F-value*	*p*
s(Exposure Length): Volume(Medium)	4.78		22.23	<0.001
s(Exposure Length): Volume(High)	1.00		2.05	0.152
s(Hour of day)	12.36		129.75	<0.001
Largemouth bass				
Intercept	−2.09	0.20	−10.22	<0.001
Volume (High)	0.95	0.13	7.36	<0.001
Speaker (B)	0.99	0.15	6.43	<0.001
Volume (High): Speaker (B)	−1.07	0.18	−5.90	<0.001
Smooth terms	*edf*		*F-value*	*p*
s(Exposure Length): Volume(Medium)	2.61		14.75	0.009
s(Exposure Length): Volume(High)	1.00		18.04	<0.001
s(Hour of day)	8.25		276.67	<0.001
Rainbow trout				
Intercept	−2.93	0.35	−8.473	<0.001
Exposure Length	−0.02	0.02	−1.41	0.160
Volume (High)	−0.24	0.12	−2.01	0.044
Speaker (B)	−0.17	0.12	1.36	0.175
Smooth terms	*edf*		*F-value*	*p*
s(Hour of day)	7.03		280.2	<0.001

[a] Estimated degrees of freedom.

ticle acceleration compared to sound pressure at low frequencies. The largemouth bass models provide evidence that near speaker detections decrease over time. However, it is also likely that the sound pressure from a single turbine would not be intense enough for detection.

The absence of hearing specialization (Halvorsen et al., 2009) and narrow frequency range sensitivities (Hawkins and Johnstone, 1978; Wysocki et al., 2007) previously published for various salmonids suggested that a response from rainbow trout to HK sound may not be detectable. Our results provide evidence that near speaker detections decreased at the highest tested volumes, suggesting the possibility of avoidance behavior when exposed to the sound emanating from a single operating turbine. Tidal turbine entrainment and blade strike has been raised as a concern for migratory fish species (Čada et al., 2007), but studies have shown high probabilities of blade avoidance and survival for a number of fish species, including rainbow trout (Amaral et al., 2015; Hammar et al., 2013). These results suggest that sound emanating from turbines might play a role in turbine avoidance, and raises the question of how cumulative sound from turbine arrays could influence migratory behavior. An important caveat when interpreting results from the rainbow trout utilized in the present study stems from observed abnormalities in the otolith structure and auditory brain response in hatchery-reared salmonids (Oxman et al., 2007; Chittenden et al., 2010; Brown et al., 2013; Wysocki et al., 2007). In addition to morphological differences, behavioral differences have also been observed between wild and hatchery salmonids (Chittenden et al., 2010) which suggests that inferences from experimental results with hatchery fish to wild stocks should be done with caution.

Given the limited number of developed HK sites, both *in situ* field studies/monitoring and further controlled studies are recommend to better assess the full range of behavioral responses of resident and migratory species to HK turbine detection cue in order to inform the site selection process and mitigation of potential ecological impacts. However, as noted by Hawkins and Popper (2016), controlled field studies of noise exposure like the one presented here can be extremely challenging. In hindsight there were a few lessons-learned during this study that would benefit future researchers who pursue similar controlled field studies. For example, the precision of our fish locating system was limited by the manufacturer-installed precision settings for the internal timer. The rapid roll-off in sound intensities, especially at the most intense treatment levels, is within the localization error (Fig. 1). Because our net pen was only 19 m long and 6 m wide, greater precision was needed to differentiate among signal times-of-arrival from different receivers. With greater precision in signal timing we could have reduced the 1D variance (±1.8 m) and likely calculated 2D location for each fish.

A second area for improvement would be more frequent detection of tags. With the exception of a few blind spots in the pen where signals might have been poorly detected, the real bottleneck in detection is related to the internal timing of interrogation by the receiver system. With the TDOA system, as the number of tags and frequencies used increases, the detection rate decreases because the system "listens" for the transmitters on a single channel of frequencies at a time. Trading off the sampling rate for

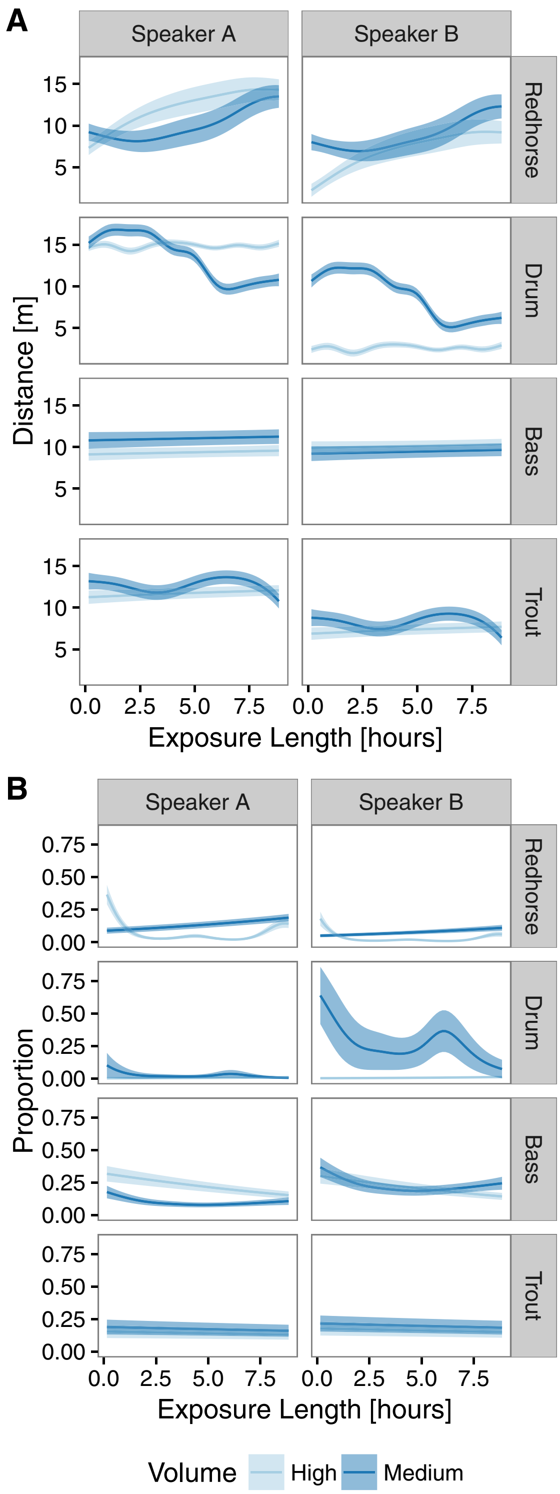

Fig. 6. Smoothed trends of (A) distance from speaker and (B) proportion of detections within 5 m of speaker based on best GAMM model for each species. Shaded areas indicate 95% confidence intervals.

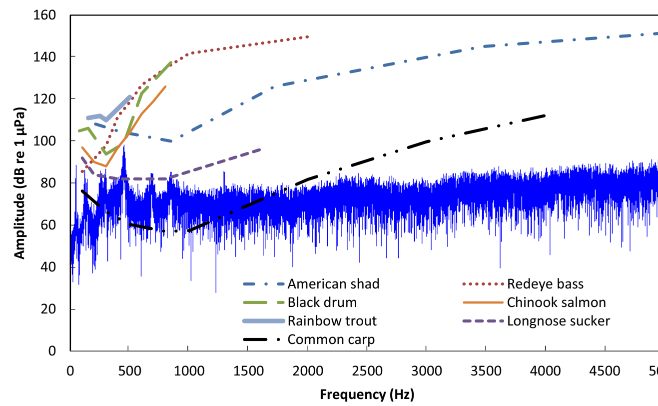

Fig. 7. Minimum hearing thresholds of American shad (*Alosa sapidissoma*), black drum (*Pogonias cromis*), Chinook salmon (*Oncorhynchus tshawytscha*), redeye bass (*Micropterus coosae*), and rainbow trout compared to the SPL produced during playback of the ORPC turbine recording at the loudest treatment level and 0.5 m distance from the speaker. Sources for hearing thresholds are Ren et al. (2012) and Wysocki et al. (2007).

increased sample size (i.e., number of tagged fish) meant each fish could only be detected every few minutes. In addition, we were not able to detect immediate reactions, such as startle responses, that might have occurred when the turbine sound was first turned on. With present technology, higher detection frequencies could have been achieved by using multiple receiver systems or by reducing the number of fish tested during a trial. In the future, more advanced electronics or smarter receiver systems will hopefully be constructed that provide more rapid signal interrogation.

Furthermore, accurately characterizing and replicating sound fields under different field and experimental conditions can present a significant challenge. Finally, detecting behavioral impacts can be challenging, especially in a field setting, due to multiple ways in which they might manifest in response to stimuli, as well as in response to stimuli outside of experimental controls.

Another technical aspect that arose during this study was that underwater speakers, despite producing the desired SPL, are not always capable of reproducing the exact spectral signature of the original recording (see Fig. 2). For the underwater speakers used here, this was particularly true for low frequencies (i.e., <400 Hz). Auditory perception at these low frequencies is an important portion of the hearing range of most fishes, however, most also perceive sound at frequencies above 400 Hz (Ladich and Fay, 2013).

Our results provide evidence that the sound pressure produced by a single HK turbine might influence the presence of fish at the tested volumes, although the effects are small. Our goal was to detect changes in behavior that would have meaningful ecological relevance. Being able to detect small, even if statistically significant, changes would not necessarily translate into meaningful ecological significance. Although our system could have been improved to detect finer responses as noted above, we believe that the system was still sufficient to detect responses at a level that would address our original goal. Even though we detected some significant responses, we did not observe the kind of overwhelming response that would portend an ecologically relevant outcome in an actual tidal turbine setting.

Our evaluation of the avoidance responses of four species of freshwater fish to sound produced by a single hydrokinetic turbine type only scratched the surface of potential effects of noise from hydrokinetic energy devices. Certainly more species, more sound sources (i.e., different types of turbines), and a larger range of volumes need to be tested. Whereas we only evaluated change in location during exposures of 1 or more hours, immediate reactions to noise upon startup should also be investigated as well as other types of behavioral responses such as changes in level of activity during sound exposure. Future studies should also consider the effects of sound in terms of particle acceleration in addition to sound pressure. Due to the lack of data characterizing sound pressure and particle acceleration produced by HK turbines, further work is also needed to assess the sound characteristics of not only individual turbines but also the cumulative sound output of turbine arrays

Acknowledgements

This research was supported by the United States Department of Energy's (DOE) Office of Energy Efficiency and Renewable Energy, Water Power Program. This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the US Department of Energy. We would like to thank Jayson Martinez and Daniel Deng (both of Pacific Northwest National Laboratory) for assistance with the underwater sound recording system. Marlin Gregor of (Sonotronics Inc.) developed the custom receiver system. Brenda Pracheil (ORNL) and Cheryl Murphy (Michigan State University) assisted with surgical implantation of tags. Scientific Solutions Inc. and Ocean Renewable Power Company provided the ORPC turbine recording. Sherree Shaw, Paul Waldschlager, and Wayne Mitchell (URS|CH2M Oak Ridge) and Mark Peterson (ORNL) facilitated access to Kerr Hollow Quarry. Samantha Eaves (DOE) reviewed an early draft of the manuscript. Jocelyn Brown-Saracino (DOE) and Shelaine Curd (ORNL) provided valuable project management oversight.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fishres.2017.01.012.

References

Čada, G.F., Ahlgrimm, J., Bahleda, M., Bigford, T., Stavrakas, S.D., Hall, D., Moursund, R., Sale, M., 2007. Potential impacts of hydrokinetic and wave energy conversion technologies on aquatic environments. Fisheries 32, 174–181, http://dx.doi.org/10.1577/1548-8446(2007)32[174:PIOHAW]2.0.CO;2.

Akamatsu, T., Okumura, T., Novarini, N., Yan, H.Y., 2002. Empirical refinements applicable to the recording of fish sounds in small tanks. J. Acoust. Soc. Am. 112, 3073–3082, http://dx.doi.org/10.1121/1.1515799.

Amaral, S.V., Bevelhimer, M.S., Čada, G.F., Giza, D.J., Jacobson, P.T., McMahon, B.J., Pracheil, B.M., 2015. Evaluation of behavior and survival of fish exposed to an axial-flow hydrokinetic turbine. North Am. J. Fish. Manage. 35, 97–113, http://dx.doi.org/10.1080/02755947.2014.982333.

Amoser, S., Ladich, F., 2005. Are hearing sensitivities of freshwater fish adapted to the ambient noise in their habitats? J. Exp. Biol. 208, 3533–3542, http://dx.doi.org/10.1242/jeb.01809.

Anderson, D.R., Link, W.A., Johnson, D.H., Burnham, K.P., 2001. Suggestions for presenting the results of data analyses. J. Wildlife Manage. 65, 373–378.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48, http://dx.doi.org/10.18637/jss.v067.i01.

Bevelhimer, M.S., Čada, G.F., Fortner, A.M., Schweizer, P.E., Riemer, K., 2013. Behavioral responses of representative freshwater fish species to electromagnetic fields. Trans. Am. Fish. Soc. 142, 802–813, http://dx.doi.org/10.1080/00028487.2013.778901.

Bevelhimer, M.S., Deng, Z.D., Scherelis, C., 2016. Characterizing large river sounds: providing context for understanding the environmental effects of noise produced by hydrokinetic turbines. J. Acoust. Soc. Am. 139 (1), 85–92, http://dx.doi.org/10.1121/1.4939120.

Biesinger, Z., Bolker, B.M., Marcinek, D., Grothues, T.M., Dobarro, J.A., Lindberg, W.J., 2013. Testing an autonomous acoustic telemetry positioning system for fine-scale space use in marine animals. J. Exp. Mar. Biol. Ecol. 448, 46–56, http://dx.doi.org/10.1016/j.jembe.2013.06.007.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H., White, J.S., 2009. Generalized linear mixed models: a guide for ecology and evolution. Trend Ecol. Evol. 24, 127–135, http://dx.doi.org/10.1016/j.tree.2008.10.008.

Brown, A.D., Sisneros, J.A., Jurasin, T., Nguyen, C., Coffin, A.B., 2013. Differences in lateral line morphology between hatchery- and wild-origin steelhead. PLoS One 8, http://dx.doi.org/10.1371/journal.pone.0059162.

Castro-Santos, T., Haro, A., 2015. Survival and behavioral effects of exposure to a hydrokinetic turbine on juvenile Atlantic salmon and adult American shad. Estuaries Coasts 38, S203–S214, http://dx.doi.org/10.1007/s12237-013-9680-6.

Chittenden, C.M., Biagi, C.A., Davidsen, J.G., Davidsen, A.G., Kondo, H., McKnight, A., Pedersen, O.P., Raven, P.A., Rikardsen, A.H., Mark Shrimpton, J., Zuehlke, B., Scott McKinley, R., Devlin, R.H., 2010. Genetic versus rearing-environment effects on phenotype: hatchery and natural rearing effects on hatchery- and wild-born coho salmon. PLoS One 5, http://dx.doi.org/10.1371/journal.pone.0012261.

Deng, Z.D., Weiland, M.A., Fu, T., Seim, T.A., LaMarche, B.L., Choi, E.Y., Carlson, T.J., Eppard, M.B., 2011. A cabled acoustic telemetry system for detecting and tracking juvenile salmon: part 2. Three-dimensional tracking and passage outcomes. Sensors 11, 5661–5676, http://dx.doi.org/10.3390/s110605661.

Fay, R.R., Popper, A.N., 2012. Fish hearing: new perspectives from two senior bioacousticians. Brain Behav. Evol. 79, 215–217, http://dx.doi.org/10.1159/000338719.

Gelman, A., Hill, J., 2007. Data Analysis Using Regression and Multilevel/Hierarchical Models. University Press, Cambridge; New York: Cambridge.

Haas, K.A., Fritz, H.M., French, S.P., Smith, B.T., Neary, V., 2011. Assessment of Energy Production Potential from Tidal Streams in the United States. Georgia Tech Research Corporation, Retrieved: http://www1.eere.energy.gov/water/pdfs/1023527.pdf.

Halvorsen, M.B., Wysocki, L.E., Stehr, C.M., Baldwin, D.H., Chicoine, D.R., Scholz, N.L., Popper, A.N., 2009. Barging effects on sensory systems of Chinook salmon smolts. Trans. Am. Fish. Soc. 138, 777–789, http://dx.doi.org/10.1577/T08-106.1.

Halvorsen, M.B., Carlson, T.J., Copping, A.E., 2011. Effects of Tidal Turbine Noise on Fish Hearing and Tissues. Pacific Northwest National Laboratory, Sequim, WA, Retrieved http://www.pnnl.gov/main/publications/external/technical_reports/PNNL-20786.pdf.

Hammar, L., Andersson, S., Eggertsen, L., Haglund, J., Gullström, M., Ehnberg, J., Molander, S., 2013. Hydrokinetic turbine effects on fish swimming behaviour. PLoS One 8, 1–12, http://dx.doi.org/10.1371/journal.pone.0084141.

Hammar, L., Eggertsen, L., Andersson, S., Ehnberg, J., Arvidsson, R., Gullström, M., Molander, S., 2015. A probabilistic model for hydrokinetic turbine collision risks: exploring impacts on fish. PLoS One 10, e0117756, http://dx.doi.org/10.1371/journal.pone.0117756.

Hawkins, A.D., Johnstone, A.D.F., 1978. The hearing of the atlantic salmon, salmo salar. J. Fish Biol. 13, 655–673, http://dx.doi.org/10.1111/j.1095-8649.1978.tb03480.x.

Hawkins, A.D., Popper, A.N., 2016. A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates. ICES J. Mar. Sci., http://dx.doi.org/10.1093/icesjms/fsw205.

Holt, D.E., Johnston, C.E., 2011. Hearing sensitivity in two black bass species using the auditory brainstem response approach. Environ. Biol. Fishes 91, 121–126, http://dx.doi.org/10.1007/s10641-010-9756-0.

Ladich, F., Fay, R.R., 2013. Auditory evoked potential audiometry in fish. Rev. Fish Biol. Fish. 23, 317–364, http://dx.doi.org/10.1007/s11160-012-9297-z.

Mann, D.A., Cott, P.A., Hanna, B.W., Popper, A.N., 2007. Hearing in eight species of northern Canadian freshwater fishes. J. Fish Biol. 70, 109–120, http://dx.doi.org/10.1111/j.1095-8649.2006.01279.x.

Martinez, J.J., Myers, J.R., Carlson, T.J., Deng, Z.D., Rohrer, J.S., Caviggia, K.A., Woodley, C.M., Weiland, M.A., 2011. Design and implementation of an underwater sound recording device. Sensors 11, 8519–8535, http://dx.doi.org/10.3390/s110908519.

Oxman, D.S., Barnett-Johnson, R., Smith, M.E., Coffin, a, Miller, D.L., Josephson, R., Popper, A.N., 2007. The effect of vaterite deposition on sound reception, otolith morphology, and inner ear sensory epithelia in hatchery-reared Chinook salmon (Oncorhynchus tshawytscha). Can. J. Fish. Aquat. Sci. 64, 1469–1478, http://dx.doi.org/10.1139/f07-106.

Pineheiro, J., Bates, D., DebRoy, S., Sarkar, D., 2015. Nlme: Linear and Nonlinear Mixed Effects Models. R package version 3, pp. 1–122 http://CRAN.R-project.org/package=nlme.

Popper, A.N., Fay, R.R., 2011. Rethinking sound detection by fishes. Hear. Res. 273, 25–36, http://dx.doi.org/10.1016/j.heares.2009.12.023.

Popper, A.N., Hastings, M.C., 2009. The effects of anthropogenic sources of sound on fishes. J. Fish Biol. 75, 455–489, http://dx.doi.org/10.1111/j.1095-8649.2009.02319.x.

Popper, A.N., 2003. Effects of anthropogenic sounds on fishes. Fisheries 28, 24–31, http://dx.doi.org/10.1577/1548-8446(2003)28[24:EOASOF]2.0.CO;2.

Ramcharitar, J., Popper, A.N., 2004. Masked auditory thresholds in sciaenid fishes: a comparative study. J. Acoust. Soc. Am. 116, 1687, http://dx.doi.org/10.1121/1.1771614.

Ramcharitar, J., Gannon, D.P., Popper, A.N., 2006. Bioacoustics of fishes of the family Sciaenidae (Croakers and Drums). Trans. Am. Fish. Soc. 135, 1409–1431, http://dx.doi.org/10.1577/T05-207.1.

Ravens, T., Cunningham, K., Scott, G., 2012. Assessment and Mapping of the Riverine Hydrokinetic Energy Resource in the Continental United States. Electric Power Research Institute, Palo Alto, CA, Retrieved: http://water.energy.gov/pdfs/riverine_hydrokinetic_resource_assessment_and_mapping.pdf.

Ren, H., Halvorsen, M.B., Deng, Z.D., Carlson, T.J., 2012. Aquatic Acoustic Metrics Interface utility for underwater sound monitoring and analysis. Sensors 12, 7438–7450, http://dx.doi.org/10.3390/s120607438.

United States Department of the Interior and United States Department of Commerce, 2012. 2011 National Survey of Fishing, Hunting, and Wildlife-associated Recreation. United States Department of the Interior, United States Fish and Wildlife Service; United States Department of Commerce, United States Census Bureau, Washington, DC, Retrieved: http://www.census.gov/prod/2012pubs/fhw11-nat.pdf.

Viehman, H.A., Zydlewski, G.B., 2014. Fish interactions with a commercial-scale tidal energy device in the natural environment. Estuaries Coasts 38, 1–12, http://dx.doi.org/10.1007/s12237-014-9767-8.
Wood, S.N., Scheipl, F., 2014. Gamm4: Generalized Additive Mixed Models Using Mgcv and Lme4. R package version 0, pp. 2–3 https://CRAN.R-project.org/package=gamm4.
Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. R. Stat. Society (B) 73, 3-36:, http://dx.doi.org/10.1111/j.1467-9868.2010.00749.x.
Wysocki, L.E., Ladich, F., 2005. Hearing in fishes under noise conditions. J. Assoc. Res. Otolaryngol. 6, 28–36, http://dx.doi.org/10.1007/s10162-004-4043-4.
Wysocki, L.E., Davidson, J.W., Smith, M.E., Frankel, A.S., Ellison, W.T., Mazik, P.M., Popper, A.N., Bebak, J., 2007. Effects of aquaculture production noise on hearing, growth, and disease resistance of rainbow trout Oncorhynchus mykiss. Aquaculture 272, 687–697, http://dx.doi.org/10.1016/j.aquaculture.2007.07.225.
Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York.