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Effects of aquaculture production noise on hearing, growth, and disease resistance of rainbow trout *Oncorhynchus mykiss*

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Abstract

Intensive aquaculture production often utilizes equipment (e.g., aerators, air and water pumps, harvesters, blowers, filtration systems, and maintenance machinery) that increases noise levels in fish culture tanks. Consequently, chronic exposure to elevated noise levels in tanks could negatively impact cultured species. Possible effects include impairment of the auditory system, increased stress, and reduced growth rates. The objective of this study was to evaluate the long-term effects of sound exposure on the hearing sensitivity, growth, and survival of cultured rainbow trout (*Oncorhynchus mykiss*). Two cohorts of rainbow trout were cultured for 8 months in replicated tanks consisting of three sound treatments: 115, 130, or 150 decibels referenced at 1 micropascal (dB re 1 μPa root mean square [RMS]) levels. Auditory evoked potential (AEP) recordings revealed no significant differences in hearing thresholds resulting from exposure to increased ambient sound levels. Although there was no evident noise-induced hearing loss, there were significant differences in hearing thresholds between the two fish cohorts examined. No statistical effect of sound treatment was found for growth rate and mortality within each fish cohort. There was no significant difference in mortality between sound treatments when fish were exposed to the pathogen *Yersinia ruckeri*, but there was significantly different mortality between cohorts. This study indicated that rainbow trout hearing sensitivity, growth, survival, stress, and disease susceptibility were not negatively impacted by noise levels common to recirculating aquaculture systems. These findings should not be generalized to all cultured fish species, however, because many species, including catfish and cyprinids, have much greater hearing sensitivity than rainbow trout and could be affected differently by noise.

Keywords: Rainbow trout; Hearing thresholds; Growth rates; Tank noise

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1. Introduction

Interest in the effects of environmental noise on fishes has grown considerably over the past several years (e.g., Popper, 2003; Popper et al., 2004). Although most of the research to date has focused on the impacts of sound sources such as sonar, pile driving, and seismic air guns that often produce sounds over 190 decibels referenced at 1 micropascal (dB re 1 μPa root mean square [RMS]) levels at the animal, there is also interest in the potential effects of exposure to chronic lower level sounds. Potential effects on fishes are likely to depend on the characteristics of the sound including level, duration, and spectrum as well as on the hearing abilities of the fish species of interest. Apparent effects could range from nondetectable, subtle behavioral changes to more dramatic physiological effects including deafness or death.

Exposure to different types of noise (pure tones or broadband white noise) at sound pressure levels (SPLs) ranging from 142 to 170 dB re 1 μPa has been shown to induce temporary hearing loss in fishes (Popper and Clarke, 1976; Scholik and Yan, 2001; Amoser and Ladich, 2003; Smith et al., 2004a), and in some cases, damage to portions of the sensory epithelia of the inner ear has been reported after exposing fishes to either pure-tone stimuli with maximum SPLs of 180 dB re 1 μPa (Enger, 1981; Hastings et al., 1996), air gun signals (main energy content from 20 to 1000 hertz [Hz]) with maximum peak-to-peak received levels of 180 dB re 1 μPa (McCauley et al., 2003), or broadband white noise of 170 dB re 1 μPa SPL (Smith et al., 2006). In addition, endocrinological stress responses to noise have been reported in Atlantic salmon (*Salmo salar*) exposed to underwater explosions of approximately 2 megapascals (MPa) in pressure amplitude (Sverdrup et al., 1994), European sea bass (*Dicentrarchus labrax*) exposed to air gun blasts developing a total volume of approximately 2500 $in.^3$ (Santulli et al., 1999), goldfish (*Carassius auratus*) within the first 10 min of exposure to broadband white noise of 170 dB re 1 μPa (Smith et al., 2004a), and in common carp (*Cyprinus carpio*), gudgeon (*Gobio gobio*), and European perch (*Perca fluviatilis*) exposed to boat noise of 153 dB re 1 μPa equivalent continuous SPL over 30 min (Wysocki et al., 2006). Increased stress levels, especially when chronic, could potentially have detrimental effects on growth, sexual maturation and reproduction, immunological function or disease susceptibility, and survival in fishes (Pickering, 1992; McCormick, 1999; Weyts et al., 1999; Pankhurst and van der Kraak, 2000; Consten et al., 2001a,b, 2002; Huntingford et al., 2006).

Not only are fishes in many natural habitats confronted with increasing levels of anthropogenic noise, cultured fishes may also be exposed to noise, especially in large, commercial-scale aquaculture facilities. Intensive aquaculture production often utilizes equipment such as aerators, air and water pumps, harvesters, blowers, and filtration systems that produce increased ambient noise levels in culture tanks, especially at low frequencies (e.g., below 1 kHz). Bart et al. (2001) found that mean broadband SPLs differed across various intensive aquaculture systems. These levels varied from <100 dB re 1 μPa in an earthen pond with the aerator turned off, 120 dB re 1 μPa RMS in concrete raceways, and 130 dB re 1 μPa in round fiberglass tanks of various sizes. In the same study, a maximum sound level of 135 dB re 1 μPa was measured in an earthen pond near an operating aerator, whereas large fiberglass tanks (14 m diameter) within a recirculating system had the highest SPLs of 153 dB re 1 μPa.

Consequently, fish in culture facilities are chronically exposed to noise levels that are well within the hearing range of many aquaculture species. However, only a few studies have investigated the effects of sound levels relative to aquaculture settings. Lagardère (1982) and Regnault and Lagardère (1983) reported that chronic elevation of in-tank noise levels (about 30 dB higher than levels encountered in their natural habitat) resulted in significant reductions in growth and reproduction rates, increased mortality, and higher metabolic rates, expressed as ammonia excretion rate and oxygen consumption in brown shrimp, *Crangon crangon.* Additionally, Banner and Hyatt (1973) observed lower egg viability and reduced growth rates of two cyprinodontiform fishes in small glass aquaria when SPLs were approximately 20 dB higher than levels in the control tanks. Although these studies are of interest, there has currently been no investigation that has examined the effects of long-term chronic noise exposure throughout the life cycle of fishes in aquaculture facilities.

The purpose of the present study was to investigate whether rainbow trout (*Oncorhynchus mykiss*) raised in tanks with different levels of noise differ in their development. The study considered the effects of increased background noise on trout hearing, growth, mortality, stress indicator constituents in the blood (glucose, sodium, and chloride), and immunocompetence (mortality following a *Yersinia ruckeri* pathogen challenge).

2. Materials and methods

2.1. Sound treatment tanks

Prior to the study, sound recordings were taken in a commercial-scale (9.1 meters [m] diameter, 2.4 m deep) round

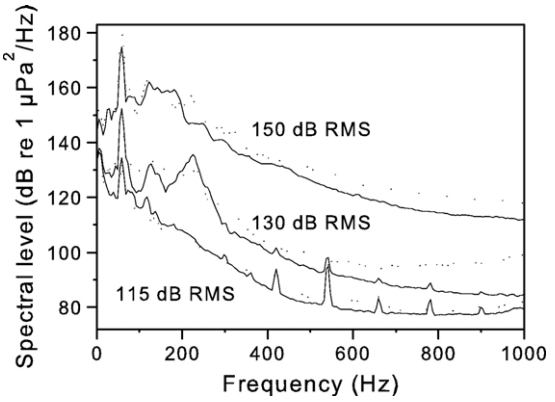

Fig. 1. Average sound density spectra of the three sound treatment tanks for cohort 1 (solid lines) and cohort 2 (dotted lines).

fiberglass aquaculture tank within a recirculating system at the Freshwater Institute (described in Summerfelt et al., 2004). Recordings were made using a hydrophone (HTI-94-SSQ; frequency response: 2 Hz to 30 kHz; sensitivity: −170 dB re 1 V/µPa; High Tech Inc., Gulfport, MS) connected to a low-pass filter set to 2000 Hz (Model 91149A, Precision Filters, Inc., Ithaca, NY), a preamplifier (Model FP-11, Shure Inc., Niles, IL), and an analog-to-digital converter and data logger (Model USB-9215, National Instruments, Austin, TX) connected to a laptop computer. Characterization of sound spectra and corresponding SPLs was performed with NIDAQmx Base Software using a Labview 7.1 application (National Instruments, Austin, TX). The SPLs in the tank averaged 130 dB re 1 µPa broadband RMS and are typical of those in other recirculating systems (Bart et al. 2001). A 5 min audio recording was created by adding tonal signals (25, 29, and 58 Hz) to the recording of the ambient noise of a quiet experimental tank to closely simulate the existing in-water sound characteristics recorded in the commercial-scale tank (Fig. 1). The recording was burned to a CD and replicated 14 times without interruption in order to play back sounds of controlled spectra and SPLs in the experimental tanks. The CD was played continuously 24 h per day. The sound was transmitted to the tanks via amplifiers (MPA-250, Radio Shack) and tactile speakers (Model AW339, Clark Synthesis Tactile Sound, Littleton, CO) mounted on the outside walls of the tanks 38 centimeters (cm) from the top and bottom of the water column.

Six round fiberglass tanks (1.5 m diameter, 0.8 m deep) within a flow-through system were used in the study. Sound levels associated with the flow-through system were relatively quiet compared to recirculating systems at the Freshwater Institute; however, oxygen saturator pumps and a carbon dioxide blower produced tonal frequencies that were transmitted into the tanks (Davidson et al., 2007). Therefore, the experimental tanks were designed to buffer ambient sound by eliminating contact between vibrating pipes and tank surfaces and by using insulated padding beneath the tanks and around the PVC pipes (Davidson et al., in press). These modifications were applied to all tanks used in the present study to limit excess background noise, to standardize tanks, and to create two relatively quiet control tanks (115 dB re 1 µPa) broadband (2 Hz to 20 kHz) RMS. Two experimental tanks had sound levels of 130 dB re 1 µPa broadband RMS and two had sound levels of 150 dB re 1 µPa RMS (Fig. 1). These treatment categories represented levels that were lower than, similar to, and higher than mean sound levels recorded within commercial-scale recirculating systems.

RMS sound levels for each tank were measured using a grid system that consisted of 15 locations: 5 horizontal (5, 38, 76, 38, and 5 cm from the sides of the tank) and 3 depths (5, 38, and 71 cm). SPL measurements were taken weekly at all locations to ensure that sound levels were consistent throughout the study without any indication of change in signal levels. Average RMS values were calculated based on the 15 locations recorded in each tank. SPLs generally varied depending on location within the tanks, with the loudest areas closest to the side walls and the bottom of the tank and the quietest locations near the top and center of the tanks (Fig. 2). In addition, a weekly 15-second (s) sound recording was taken 38 cm from the side of the tanks at a depth of 38 cm to ensure that the spectral composition of the noise remained consistent over time.

2.2. Animals

Fertilized rainbow trout eggs (*O. mykiss*) were obtained from a commercial fish hatchery (Troutlodge, Sumner, WA). All gametes originated from three-year-old parents from the same gene pool. The zygotes were all female diploids and a cross between rainbow trout (the stationary freshwater form of *O. mykiss*) and steelhead trout (the anadromous form of *O. mykiss*). The fertilized eggs were shipped overnight and received at the Freshwater Institute at 3 °C. They were acclimated to hatching system temperatures (13 °C) over a 2-hour (h) time period and

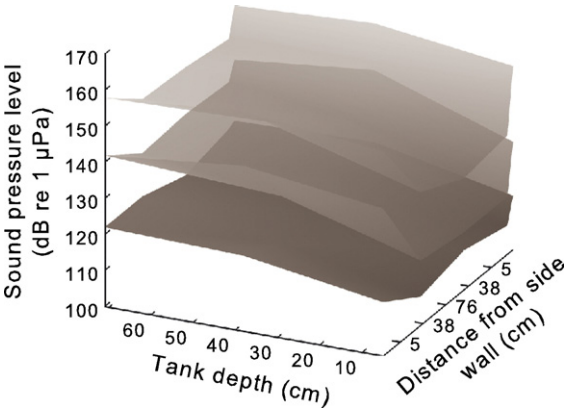

Fig. 2. Variability of sound pressure levels (dB re 1 µPa broadband [2 Hz to 20 kHz] RMS) within the different sound treatment tanks. Sound levels represent mean RMS measurements taken at various locations within each sound treatment tank for cohort 1 and cohort 2 over the course of the study.

then divided into trays within the hatching system. Day 1 of the life cycle (as reference for all following age specifications) was designated 6 days after arrival when 50% of the eggs had hatched. On day 15, fish were stocked into a single round tank (1.5 m diameter, 0.8 m depth). The mean ambient noise level in this tank was 115 dB re 1 μPa broadband RMS. Water depth was gradually increased as fish grew, to a maximum of 0.8 m depth. On day 84, fish were divided into 2 tanks with the same dimensions and SPLs to reduce fish density. The study officially began on day 92 when fish from the 2 tanks were randomly divided into 3 sound treatment tanks (115, 130, and 150 dB re 1 μPa RMS) at a density of 700 fish per tank or 10 kilograms per cubic meter (kg/m^3). The mean weight at stocking for all tanks was 14±0.1 grams (g).

The second cohort of trout, from the same batch of eggs that provided fish in cohort 1, was also stocked into respective sound treatment tanks (115, 130, and 150 dB re 1 μPa RMS) on day 92 of the life cycle and underwent exactly the same procedures and treatments at comparable ages as the above-described cohort 1 animals, with the only difference that the fertilized eggs were received and hatched 3 weeks later. Note: 2 cohorts of rainbow trout were evaluated to provide replication and were received at different times to facilitate the logistics of fish hearing tests.

Throughout the study, all fish were cultured under a constant 24-h photoperiod at 12.5 to 13.5 °C in a flow-through system. They were fed slow-sinking trout feed (Zeigler Brothers Inc., Gardners, PA) with a protein-to-fat ratio of 42:16 via automated feeders (Sterner Products AB, Leksand Sweden) that were programmed and calibrated weekly to deliver the same amount of feed to each tank. By the end of the study, trout had reached market size; thus the duration of the study was representative of the period that rainbow trout are typically cultured.

2.3. Auditory threshold determination

Auditory thresholds were determined by recording auditory evoked potentials (AEPs), often called auditory brain stem response (ABR). This response is the combined electrical output of the inner ear and auditory brain stem and reflects the responses of this peripheral part of the auditory system to acoustic signals (Kenyon et al., 1998; Smith et al., 2004a,b). AEPs were measured at 3 different ages: 1) age 17 and 16 weeks for cohorts 1 and 2, respectively ($n=10$ per sound treatment; $n=20$ per cohort), 2) age 33 and 32 weeks for cohorts 1 and 2, respectively ($n=10$ per sound treatment; $n=20$ per cohort), and 3) age 41 and 38 weeks for cohorts 1 and 2, respectively ($n=6$ per sound treatment; $n=12$ per cohort). Hearing tests were conducted at slightly different ages for the different cohorts in order to facilitate logistics of testing by enabling sampling of both cohorts on the same day. Moreover, the main purpose of the third experimental series was to test whether auditory threshold differences between cohorts detected during the previous test series reflected differences in hearing between cohorts or just variations in experimental set up between test dates. Therefore, the third series of auditory threshold determination was conducted for fish from both cohorts on the same date rather than again at comparable ages but two weeks apart. During the third series of hearing sensitivity tests, fish from both cohorts were measured on the same days to test for differences in hearing sensitivity between cohorts.

During the hearing tests, the fish were mildly immobilized with an intramuscular injection of Flaxedil (gallamine triethiodide, Sigma) at doses of 5.8±0.5 micrograms per gram ($\mu g\ g^{-1}$) body mass for the 15- to 16-week-old fish and 8.3±0.2 $\mu g\ g^{-1}$ body mass for the 32- to 41-week-old fish. This dosage allowed the fish to retain slight opercular movement during the experiments without creating significant myogenic noise to interfere with the AEP recordings.

Test subjects were secured in the center of a rectangular plastic tub (51×41 cm; water depth: 25 cm) that had a 4-cm-thick layer of fine gravel on the bottom to dampen vibrations of the bottom caused by the motion of the underwater speaker. Fish were restrained in a mesh sling and suspended so that the top of the head was 6 cm below the water surface. A pipette was inserted into the mouth and provided water from a simple temperature-controlled (13±1 °C), gravity-fed water circulation system.

The AEPs were recorded using stainless steel electrodes (Rochester Electro-Medical, Inc., Tampa, FL). The recording electrode was placed in the midline of the skull over the medulla region and the reference electrode was placed cranially between the nares. Both electrodes were inserted approximately 2 millimeters (mm) subdermally. All exposed surfaces of the electrode tips that were not in direct contact with the fish were insulated with fingernail polish. A ground electrode was placed in the water.

Sound stimuli presentation and AEP waveform recordings were performed with a modular rack-mount system (TDT System 3, Tucker-Davis Technologies, Gainesville, FL) and TDT BioSig RP software. Sounds were created using TDT SigGen RP software and fed through a power amplifier (Alesis RA 150, Cumberland, RI) connected to an underwater speaker (UW-30, University Sound, Burnsville, MN) placed in the center of the plastic tub. Sound stimuli were presented as repeated tone bursts at a rate of 20 per second. Hearing thresholds were determined at frequencies of 150, 250, 300, and 500 Hz, presented in random order. The duration of sound stimuli was 15 ms for 150 and 250 Hz and 10 ms for the other frequencies. Rise and fall times were 2 ms. All tone bursts were gated using a Blackman window, an acoustic filter that gives the signal a slow onset and cutoff and reduces the generation of side lobes in their frequency spectrum.

Absolute SPLs were measured using a hydrophone (10 CT; frequency response: 30 Hz to 100 kHz, ±3 dB; receiving sensitivity: −211 dB±3 dB re 1 V/μPa, G.R.A.S., Holte, Denmark) and a Kistler dual-mode amplifier (5010, Amherst, NY) at the position where the fish was placed in the test tub. For each test condition, stimuli were presented at opposite polarities (180° phase shifted), and the corresponding AEP traces were averaged by the Bio-Sig RP software in order to eliminate stimulus artifacts. Up to 500 responses were averaged for each stimulus level and polarity. SPLs of tone-burst stimuli were reduced in 5-dB steps until the AEP waveform was no longer apparent. The lowest SPL for which a repeatable AEP trace could be obtained, as determined by

overlaying replicate traces, was considered the threshold (Kenyon et al., 1998; Smith et al., 2004a). After determination of hearing thresholds, each fish was euthanized with 200 milligrams per liter (mg/l) tricaine methanesulfonate (MS-222), the inner ears were removed, and the saccular otolith type was recorded for each individual.

For technical reasons, hearing thresholds are given in terms of sound pressure, dB re 1 μPa (RMS). Because rainbow trout do not possess accessory hearing structures that enhance detection of the pressure component of sound, they are most likely primarily sensitive to particle motion. As a consequence, the thresholds presented here should not be interpreted as absolute values. However, because our main interest was to investigate if there are relative differences in detection of the same stimulus between animals raised under different conditions at different ages, use of a measure of pressure is a valid approach for purely comparative purposes.

2.4. Growth rates and blood parameter assessment

Weights and fork lengths of trout from each sound treatment and cohort were measured every 2 weeks, and dead fish (from natural causes) were collected and recorded daily. Blood samples were collected from 35 fish from each sound treatment from cohorts 1 and 2 on days 309 (week 44) and 315 (week 45), respectively. Preliminary samples indicated that, for each parameter, 35 samples would be sufficient so that the 95% confidence interval for the difference between 2 means would have a precision equal to one-half of the standard deviation for each mean (Motulsky, 1995). All blood samples for fish within the respective cohorts were collected on the same day. Fish were netted individually and sedated by immersion in 75 mg/l MS-222, and 1 ml of blood was drawn from the caudal vein within 45 s after introduction to the anesthetic. Samples were centrifuged at 2400 *g* for 5 min at 14 °C. Plasma was stored at −15 °C for less than 30 days. Chloride, sodium, and glucose concentrations as indicators of the secondary stress response were analyzed using a Hitachi 917 Chemistry Instrument (Roche Diagnostics, Indianapolis, IN; Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY).

2.5. Y. ruckeri challenge

On days 195 (cohort 1) and 197 (cohort 2), 15 fish from each sound treatment (a total 45 fish per cohort) were exposed to a pathogen challenge to determine if sound level affected resistance to enteric redmouth disease in rainbow trout. Due to biosecurity restrictions related to the potential transmission of the pathogen within the Freshwater Institute, this pathogen challenge took place at the USDA Agricultural Research Service, National Center for Cool and Coldwater Aquaculture (NCCWA), (Leetown, WV) 15 miles from the Freshwater Institute. Fifteen fish from each sound treatment tank were fin clipped for identification and transported to NCCWA. Because fish from all sound treatments were transported at the same time, transportation provided an equal stressor to all fish. Fish were stocked into a single rectangular fiberglass culture tank (61 cm diameter, 38 cm deep) with a mean sound level of 113 dB re 1 μPa RMS. Fish were then immersion challenged with *Y. ruckeri* at a concentration of 10^9 CFU/ml of tank water. After a 1-h immersion, the tanks were returned to normal flow-through conditions. Dead fish were removed daily, and fin clips were identified to determine the respective sound treatment categories.

2.6. Statistical analyses

All data sets were tested for normal distribution using the Kolmogorov–Smirnov test. Hearing thresholds between trout of the same cohort raised in 115 dB re 1 μPa tanks and the 150 dB re 1 μPa tanks were compared at each age and frequency using unpaired *t*-tests. It should be noted that additional measurements were not performed on trout from the 130 dB re 1 μPa tanks due to the similarities in data between the 130-dB and 150-dB tanks. Similarly, hearing thresholds of cohort 1 and 2 trout of the oldest age group measured on the same days were compared at each frequency using unpaired *t*-tests. Audiograms of trout from both cohorts at the three different ages were compared by a two-way analysis of variance (ANOVA) using a general linear model where one factor was age and the other was frequency. The age factor alone is an indicator for overall differences in the audiograms of the animals at different ages, and in combination with the frequency factor, it indicates whether such differences are the same throughout the whole audiogram or whether the thresholds at the various frequencies changed differently. To determine at which frequency thresholds differ, separate one-way ANOVAs followed by Scheffé's multiple-comparison procedure were calculated. The levels of statistical significance were adjusted to the number of frequencies tested ($*P \leq 0.0125$, $**P \leq 0.0025$, $***P \leq 0.00025$). Blood parameters between the different experimental groups within each cohort were compared using Kruskal–Wallis tests followed by Mann–Whitney *U* tests because the data were not normally distributed and variances were unequal. All above-mentioned statistical tests were performed using SPSS 12.0 (SPSS Inc., Chicago, IL).

Growth rates were compared using a combination of one-way ANOVA and polynomial regression using SAS version 9.1 (SAS Institute Inc., Cary, NC). Pathogen challenge mortality was analyzed using a χ^2 goodness of fit test with a log rank test for equality of survivor functions using Intercooled Stata, version 8.2. (StataCorp, College Station, TX).

3. Results

3.1. Hearing sensitivity and otolith distribution

Hearing thresholds of trout within cohort 1 between control (115-dB) and 150-dB tanks did not differ at any frequency over the duration of the experiment, and the same was found for cohort 2 trout (unpaired *t*-tests, $P > 0.05$ for all frequencies; Fig. 3A–C). Thus, there appeared to be no effect on hearing

when comparing fish exposed to increased sound levels to control animals, even as the duration of exposure increased.

Hearing was also examined within cohorts to determine whether there are developmental changes in sensitivity with age. The thresholds of fish in cohort 1 animals did not change from 16 to 41 weeks of age (two-way ANOVA: $F_{2,222}=0.76$, $P=0.47$). In contrast, hearing thresholds of cohort 2 trout were significantly different at the various ages tested ($F_{2,208}=23.6$, $P\leq 0.001$), and there was a significant interaction between age and frequency, indicating that the amount of threshold change differed between frequencies ($F_{6,208}=2.57$, $P\leq 0.001$). Further analysis indicated that hearing thresholds at 250 and 300 Hz were higher in the 33- and 38-week-old fish than in the 17-week-old group.

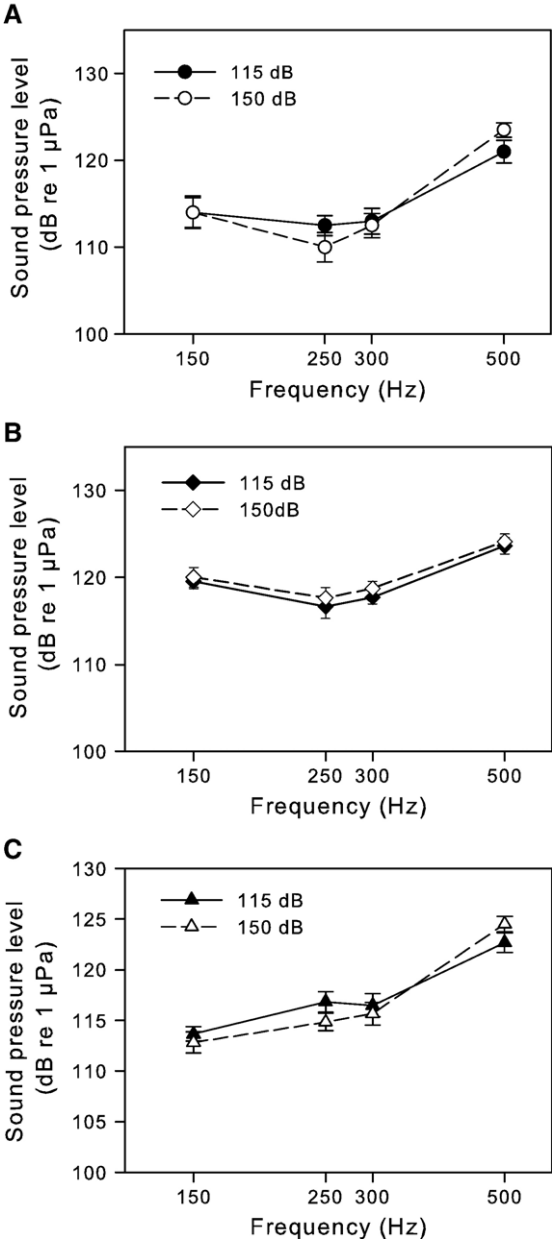

Fig. 3. Hearing thresholds of cohort 2 trout raised in 115-dB tanks (solid lines) and in 150-dB tanks (dashed lines) at the age of 16 weeks (A), 32 weeks (B), and 38 weeks (C).

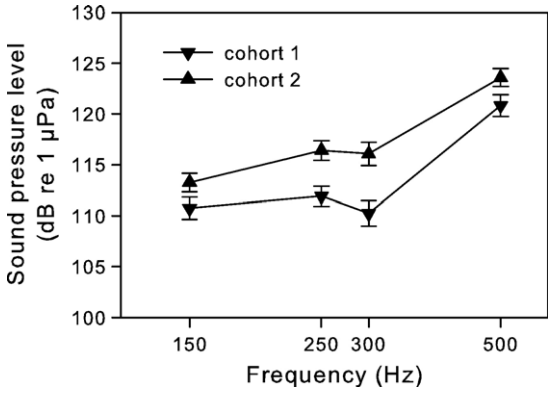

Fig. 4. Hearing thresholds of cohort 1 trout (age 41 weeks) and cohort 2 trout (age 38 weeks) measured on the same test days. Significant differences between both cohorts: *$P\leq 0.0125$; **$P\leq 0.0025$.

To confirm the differences in hearing sensitivity between cohort 1 and cohort 2 and to rule out that the differences found were due to variations in setup at different dates, hearing thresholds from both cohorts were tested on the same experimental day in a third test series regardless of the age difference of three weeks between both cohorts. The sensitivity of the two groups differed significantly at 250 and 300 Hz (unpaired *t*-tests: $P=0.004$ and $P=0.002$, respectively; Fig. 4). Cohort 2 trout had higher hearing thresholds at 250 and 300 Hz, which had already been the case when cohorts were 16 to 17 weeks old ($P\leq 0.001$ for both frequencies).

Inspection of the saccular otoliths from the tested animals showed that they had two types of otoliths, vaterite and aragonite. However, the type was not always the same for both otoliths for an individual fish. Most fish (69% of those sampled) had vaterite-type otoliths in both sacculi, 26% had one aragonite- and one vaterite-type otolith, and only 5% had aragonite-type otoliths in both sacculi. The percentage of vaterite–vaterite sacculi was compared between sound treatments and cohorts. There were relatively small

Table 1
Percent distribution of various saccular otolith crystalline structures from fish that were tested for hearing thresholds

Age	Cohort	Otolith type		
		AA	AV	VV
17 weeks	Cohort 1	4.8%	9.5%	85.7%
16 weeks	Cohort 2	20%	30%	50%
33 weeks	Cohort 1	–	10.5%	89.5%
32 weeks	Cohort 2	–	55%	45%
41 weeks	Cohort 1	8.3%	33.3%	58.3%
38 weeks	Cohort 2	–	8.3%	91.7%

AA — both ears aragonite-type saccular otoliths.
AV — saccular otolith of one ear aragonite and of the other ear vaterite.
VV — both ears vaterite-type saccular otoliths.

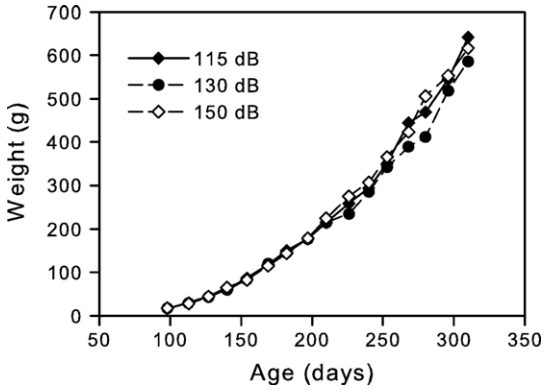

Fig. 5. Growth curves of cohort 1 trout raised in the three different sound treatment tanks.

differences in the occurrence of vaterite–vaterite sacculi between fish cultured at 115 dB and 150 dB within each cohort and age group (1–17% difference). A larger difference in vaterite–vaterite otolith distribution was found between cohorts of the same age group (16–50%) (Table 1). When comparing otolith distribution among cohorts for 16- to 17-week-old and 32- to 33-week-old fish, cohort 1 animals had a higher mean percent vaterite–vaterite distribution (87.5%) than cohort 2 animals (50%). However, this trend was reversed for the 38- to 41-week-old fish (58.5% for cohort 1, 91.5% for cohort 2).

3.2. Growth rates and mortality

Differences in growth rates between sound treatments and cohorts were analyzed using a combination of ANOVA and polynomial regression. The treatment × day interaction tested whether trends across days were the same for each treatment. Results indicated that there was no significant difference between sound treatments (Fig. 5; $F_{2,81}=0.60$, $P=0.6267$). Additionally, there was no significant difference in growth between cohorts ($F_{1,81}=3.88$, $P=0.1887$). There was a significant difference between days ($F_{1,81}=4.98$, $P=0.0284$), but this is an expected difference because the fish are continuously growing. There was no significant interaction between treatment × day ($F_{2,81}=0.73$, $P=0.4872$), indicating that there were no significant differences in the growth curves between treatments.

One-way ANOVA tests showed that there were no significant differences in overall survival (Table 2) between treatments ($F_{2,6}=3.93$, $P=0.145$) or between cohorts ($F_{1,6}=1.52$, $P=0.285$).

Table 2
Percent survival of trout from each sound treatment tank during the entire study period

	Sound treatment		
	115 dB re 1 μPa	130 dB re 1 μPa	150 dB re 1 μPa
Cohort 1	99.1%	97.7%	98.5%
Cohort 2	98.4%	96.9%	97.8%

3.3. Blood chemistry and bacterial challenge

Chloride concentrations did not differ significantly between sound treatments in either cohort (Fig. 6A; cohort 1: $\chi^2_{2,105}=3.07$, $P=0.215$; cohort 2: $\chi^2_{2,105}=3.51$, $P=0.173$). Similarly, the sodium concentrations did not differ between treatment groups for cohort 2 (Fig. 6B; $\chi^2_{2,105}=2.85$, $P=0.24$). However, sodium concentrations for cohort 1 were significantly

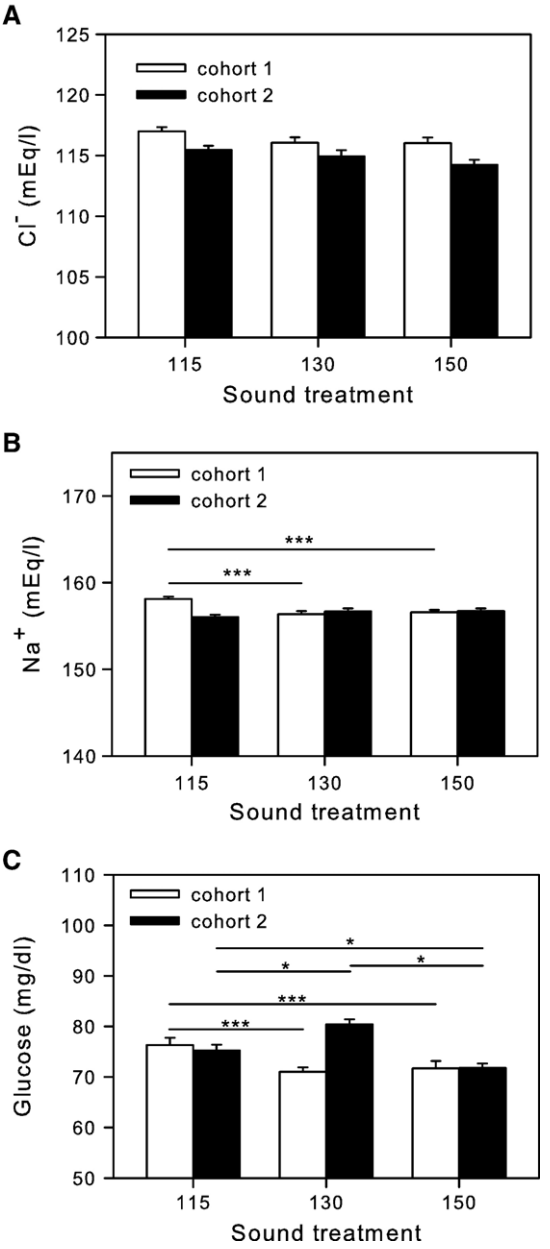

Fig. 6. Chloride (A), sodium (B), and glucose (C) blood concentrations of trout from cohort 1 (white bars) and cohort 2 (black bars) trout raised in different sound treatment tanks. Significant differences between both cohorts: $^*P \leq 0.05$; $^{**}P \leq 0.01$.

different between treatment groups ($\chi^2_{2,105}=19.64$, $P<0.001$). Subsequent Mann–Whitney U tests revealed that sodium concentrations in fish from the 115-dB treatment were significantly higher than from the other sound treatments ($P<0.001$ for the 115-dB treatment vs. the 130-dB and 150-dB treatments). Glucose levels were significantly different between sound treatment groups in both cohorts (Fig. 6C; cohort 1: $\chi^2_{2,105}=8.49$, $P=0.014$; cohort 2: $\chi^2_{2,105}=30.52$, $P<0.001$). Subsequent Mann–Whitney U tests showed that fish from cohort 1, 115-dB treatments had significantly higher glucose levels than the other sound treatment groups ($P<0.001$). In cohort 2, glucose levels differed between all treatment groups ($P<0.05$ for all three combinations) with animals from the 130-dB group having the highest values.

Mortalities resulting from the pathogen challenge did not differ significantly between sound treatment levels ($\chi^2_{2,45}=1.89$, $P=0.3886$), indicating that the sound levels that rainbow trout were subjected to during the study did not increase disease susceptibility. A significant difference in pathogen challenge mortality was detected between cohorts ($\chi^2_{2,90}=8.21$, $P=0.0042$). Cohort 1 had lower total mortality than cohort 2 (19 and 32 mortalities, respectively).

4. Discussion

4.1. Auditory thresholds

No difference in auditory thresholds was found between rainbow trout reared in 115-dB tanks and 150-dB tanks for either cohort throughout the entire 6-month observation period. Therefore, chronic exposure to noise levels and sound spectra similar to those produced within recirculating aquaculture systems did not affect the rainbow trout auditory system. Rainbow trout do not possess specialized accessory hearing structures and generally have a limited hearing bandwidth and sensitivity (such species are often called hearing "generalists") compared to species with hearing-specialized auditory peripheral structures (hearing "specialists") such as carp and catfish (Popper and Fay, 1973; Hawkins, 1993; Ladich and Popper, 2004).

Data from previous studies confirm that fish species with different overall hearing sensitivity are affected differently by exposure to noise of a given level. Effects range from substantial temporary hearing loss to no effect at all. Substantial noise-induced temporary hearing loss (ranging from 3 days to 2 weeks) of up to 30 dB has been described in several hearing-specialist fish species possessing a direct connection between the swim bladder and the inner ear (Weberian apparatus), which increases hearing sensitivity and expands hearing bandwidth relative to species without such specializations (Scholik and Yan, 2001; Amoser and Ladich, 2003; Smith et al., 2004a). In contrast, "hearing generalists," species with lower hearing sensitivity as well as a smaller hearing bandwidth, showed no or minimal hearing loss when exposed to comparable noise (Scholik and Yan, 2002; Smith et al., 2004b). Therefore, although rainbow trout were not affected by the noise levels (i.e., noise from recirculating tank) used in the current study, it is possible that other aquaculture species with more sensitive hearing, such as catfish or carp, could be impacted by noise from recirculating tanks within aquaculture facilities. Additionally, the natural habitats of rainbow trout have relatively high ambient noise levels (Lugli and Fine, 2003). SPLs in creeks and streams are usually above 110 dB re 1 μPa (equivalent continuous broadband level over 1 min), whereas ambient noise levels in stagnant habitats with high percentages of hearing-specialized fish species such as backwaters and lakes are typically below 100 dB re 1 μPa (Wysocki et al., 2007).

Although higher background noise levels did not result in changes in hearing sensitivity with increasing age throughout the study, there was a difference in hearing sensitivity developing with increasing age between cohorts. Hearing thresholds of cohort 1 animals did not change significantly with age except for a higher (but not consistent) sensitivity at 400 Hz in the 33-week-old fish. In contrast, hearing sensitivity of cohort 2 animals changed significantly with age. After 38 weeks of age, cohort 2 trout had significantly higher hearing thresholds at 250 and 300 Hz than cohort 1 trout (41 weeks). Although the relevant sound characteristic for hearing in salmonids is primarily particle motion and not sound pressure (Hawkins and Johnstone, 1978), this relative difference is between animals receiving the same sound stimulus and thus independent of the proportion between sound pressure and particle motion as well as of the absolute hearing sensitivity to particle motion. At the same time, because this experiment did not determine whether fish were responding to pressure or particle motion, it is not possible to compare data from this study to data for young or adult salmonids of any other study. Moreover, because there are no data on hearing in adult rainbow trout, it is not possible to extrapolate from our data to older animals.

Both cohorts were treated identically throughout the study including (a) same type and amount of food, (b) same water temperatures and water chemistry, and (c) rearing under identical noise regimens. Therefore, culture conditions and fish husbandry are not a likely cause for the differences observed between cohorts. Additionally, fish originated from the same genetic pool according to the fish supplier. This could not be demonstrated conclusively; therefore, genetic variance could account for the variable development in hearing sensitivity between cohorts.

Several studies have shown the occurrence of different crystalline forms of otoliths within the same population of salmonids. Although the aragonite form of calcium carbonate (composed of crystals embedded within a protein matrix) is considered the "wild type," vaterite-type (a crystalline form of calcium carbonate) otoliths were found in much higher percentages in hatchery-reared juvenile coho salmon (Sweeting et al., 2004), and there is recent evidence that auditory thresholds and otolith type correlate for Chinook salmon (Dion Oxman, personal communication). In the present study, otolith types differed between rainbow trout cohorts; however, trends within cohorts were not consistent throughout the study. For example, at 32–33 weeks old, the vaterite–vaterite type was much more abundant in cohort 1, and at 38–41 weeks old, when fish from both cohorts were tested simultaneously, vaterite–vaterite otoliths were more abundant in cohort 2. Therefore, threshold differences could not be conclusively linked to otolith type. In addition, the limited number of aragonite–aragonite otoliths collected was not sufficient to quantitatively compare differences between cohorts. Interestingly, significant differences between cohorts were not only detected for hearing thresholds but also for pathogen challenge mortality and blood chemistry constituent concentrations.

One factor that could not be controlled was the handling of the fertilized eggs during and before shipping until arrival at the Freshwater Institute. Fertilized eggs are commonly shipped on ice in coolers to maintain optimal temperatures and to prevent accelerated development. Because our trout presumably came from parents of the same gene pool but arrived 3 weeks apart at the study site, it is possible that cohort 2 eggs were chilled for a longer period than cohort 1 eggs. Thermal control is widely used in aquaculture as a means of influencing the hatching time of the larvae. A recent study on juvenile steelhead trout found evidence that cryopreservation of milt can lead to differences in weight and length as well as in overall stress levels relative to siblings produced from untreated milt (Hayes et al., 2005). Similarly, several factors in the environment of fish eggs, such as the surrounding temperature and the speed of development between fertilization and hatching, could impact the later development of the fish, and this is certainly a topic that needs further investigation.

4.2. Health parameters

Although elevated noise levels could potentially impair various health parameters, e.g., reduced growth rates, increased aggression, and reduced food uptake but higher metabolic rates such as observed in brown shrimp (Lagardère, 1982; Regnault and Lagardère, 1983), significant differences in growth rates were not detected between trout reared under the noise regimens used during this study.

Decreases in chloride and sodium plasma concentrations as well as increases in plasma glucose concentrations are part of the secondary stress response in teleost fishes (Barton and Iwama, 1991; Wendelaar Bonga, 1997). Chloride concentrations were not different between fish subjected to different sound treatments in either cohort nor were sodium concentrations in cohort 2. In cohort 1, however, fish reared in the 130- and 150-dB tanks had significantly lower sodium concentrations compared to fish reared in the 115-dB tank. The significantly lower sodium levels in fish from the 130- and 150-dB tanks could indicate that these fish were more stressed by these noise conditions compared to fish in the 115-dB tank because freshwater fishes tend to overhydrate to compensate for stress, which results in dilution of ions such as sodium in the blood. Glucose concentrations between sound treatments differed within both cohorts, although there was no common trend. For example, for cohort 1, fish from the 115-dB tank had the highest glucose concentrations among treatments and cohort 2 fish from the 130-dB tanks had the highest glucose concentrations. Blood chemistry data and analysis did not result in specific trends to conclude that elevated noise levels in the rearing tanks presented a chronic stressor to the fish.

Noise has the potential to induce stress responses in the few fish species that have been studied (Smith et al., 2004a; Wysocki et al., 2006). However, research suggests that induction of a stress response could depend on the type of noise. For example, boat engine noise, which was variable in level, time, and frequency domains, elicited increased secretion of the primary stress hormone cortisol in three species of European freshwater fishes with different hearing sensitivities. In contrast, continuous Gaussian noise of comparable intensity did not elicit a stress response in the same individuals (Wysocki et al., 2006). It has been speculated that fishes are able to habituate to a continuous stimulus. The ability to adapt to continuous noise is important because many fish species, e.g., gobies and trout, live and reproduce in inherently noisy natural habitats such as rocky creeks, torrents, and seashores (Lugli and Fine, 2003) and must therefore be able to maintain their normal activities despite high levels of background noise. The noise encountered during this study and in aquaculture tanks in general is also continuous.

Therefore, the rainbow trout could have adapted to the noise presented in this study over time.

5. Conclusions

Current typical levels of noise in aquaculture production systems are unlikely to be a limiting factor affecting growth, health, and hearing ability for rainbow trout at least up to 9 months of age. Variability in hearing sensitivity and disease resistance between different groups of trout could be related to factors such as genetic variability or egg treatment. Although rainbow trout were not affected by the noise levels used in this study, these results should not be generalized to all cultured fish species. "Hearing-specialist" species such as catfish or carp could be affected differently, especially in their sensory development. Due to the high diversity of hearing abilities and other environmental adaptations in fishes, more data are needed to confirm potential effects of noise in aquaculture facilities on other fish species.

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