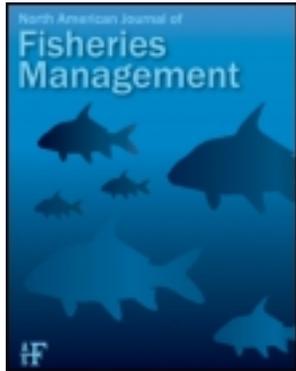


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Benjamin S. Cox^{a e}, Andrew M. Dux^b, Michael C. Quist^c & Christopher S. Guy^d

^a Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho, Post Office Box 441141, Moscow, Idaho, 83844, USA

^b Idaho Department of Fish and Game, 2885 West Kathleen Avenue, Coeur d'Alene, Idaho, 83815, USA

^c U.S. Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, Post Office Box 441141, Moscow, Idaho, 83844, USA

^d U.S. Geological Survey, Montana Cooperative Fishery Research Unit, Post Office Box 173460, Bozeman, Montana, 59717, USA

^e Oregon Department of Fish and Wildlife, 17330 Southeast Evelyn Street, Clackamas, Oregon, 97015, USA

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MANAGEMENT BRIEF

Use of a Seismic Air Gun to Reduce Survival of Nonnative Lake Trout Embryos: A Tool for Conservation?

Benjamin S. Cox*¹

Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho, Post Office Box 441141, Moscow, Idaho 83844, USA

Andrew M. Dux

Idaho Department of Fish and Game, 2885 West Kathleen Avenue, Coeur d'Alene, Idaho 83815, USA

Michael C. Quist

U.S. Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, Post Office Box 441141, Moscow, Idaho 83844, USA

Christopher S. Guy

U.S. Geological Survey, Montana Cooperative Fishery Research Unit, Post Office Box 173460, Bozeman, Montana 59717, USA

Abstract

The detrimental impacts of nonnative lake trout *Salvelinus namaycush* in the western USA have prompted natural resource management agencies in several states to implement lake trout suppression programs. Currently, these programs rely on mechanical removal methods (i.e., gill nets, trap nets, and angling) to capture subadult and adult lake trout. We conducted a study to explore the potential for using high-intensity sound from a relatively small (655.5 cm³ [40 in³]) seismic air gun to reduce survival of lake trout embryos. Lake trout embryos at multiple stages of development were exposed to a single discharge of the seismic air gun at two depths (5 and 15 m) and at two distances from the air gun (0.1 and 2.7 m). Control groups for each developmental stage, distance, and depth were treated identically except that the air gun was not discharged. Mortality in lake trout embryos treated at 0.1 m from the air gun was 100% at 74 daily temperature units in degrees Celsius (TU°C) at both depths. Median mortality in lake trout embryos treated at 0.1 m from the air gun at 207 TU°C (93%) and 267 TU°C (78%) appeared to be higher than that of controls (49% and 48%, respectively) at 15-m depth. Among the four lake trout developmental stages, exposure to the air gun at 0.1 m resulted in acute mortality up to 60% greater than that of controls. Mortality at a distance of 2.7 m did not appear to differ from that of controls at any developmental stage or at either depth. Our results indicate that seismic air guns have potential as an alternative tool

for controlling nonnative lake trout, but further investigation is warranted.

Nonnative species (i.e., introduced and invasive) are the second leading cause of anthropogenic environmental change and biodiversity loss worldwide (Vitousek et al. 1997; Wilcove et al. 1998). In the western USA, nonnative lake trout *Salvelinus namaycush* have caused declines in populations of native fish species. Predation by lake trout has been implicated in the decline of the Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* in Yellowstone Lake, Yellowstone National Park (Koel et al. 2005), and competition with nonnative lake trout has been cited in the decline of native bull trout *Salvelinus confluentus* populations in the upper Columbia River basin (Donald and Alger 1993; USFWS 1998; Fredenberg 2002). In ecosystems of the western USA, the effects of nonnative lake trout extend beyond the fish community. Zooplankton and phytoplankton assemblages have been altered by trophic cascades induced by nonnative lake trout (Tronstad et al. 2010; Ellis et al. 2011). Nonnative lake trout have also altered linkages between terrestrial and aquatic food webs by causing declines in the migratory fish populations that are preyed upon by birds and

*Corresponding author: benjamin.s.cox@state.or.us

¹Present address: Oregon Department of Fish and Wildlife, 17330 Southeast Evelyn Street, Clackamas, Oregon 97015, USA.

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mammals during spawning runs (Spencer et al. 1991; Koel et al. 2005).

The detrimental impacts of nonnative lake trout in the western USA have prompted natural resource management agencies to implement lake trout suppression programs (Martinez et al. 2009). Currently, these programs rely on mechanical removal methods (i.e., gill nets, trap nets, and angling) to capture subadult and adult lake trout. Perturbation analyses of matrix population models for lake trout in Swan Lake, Montana, suggest that lake trout population growth is highly sensitive to changes in survival from age 0 to age 1 (Cox 2010). Thus, alternative control methods that target embryonic lake trout could be an effective supplement to current mechanical removal techniques.

Use of high-intensity sound from a seismic source is a potential technique for reducing survival of early life history stages of nonnative fishes. Seismic air guns were developed in the 1960s to replace dynamite as a sound source for geophysical exploration (Giles 2009). Typical seismic air guns essentially consist of two chambers that are pressurized by air and that are sealed by a piston; an electric solenoid valve on one chamber serves as a trigger mechanism (Hutchinson and Detrick 1984). As the solenoid releases air from one chamber, hydrostatic pressure between the chambers and the environment produces an explosion, causing firing of the piston and creating an acoustic signal (Hutchinson and Detrick 1984). Increased demand and exploration (i.e., seismic surveys) for offshore petroleum have raised concerns about the effects of human-generated sound on marine life (Popper and Hastings 2009). A relatively small body of literature has examined the effects of seismic air guns on survival of embryonic marine fish and invertebrates (Hassel et al. 2004; Payne 2004; Payne et al. 2009). The effects of high-intensity sound on lake trout embryos have yet to be studied.

The objective of this study was to explore the potential for using a relatively small seismic air gun (i.e., practical for use by a small crew) as a means of reducing the survival of lake trout embryos. The effect of a seismic discharge on embryonic lake trout probably depends on several factors, including sound intensity, the developmental stage of the embryo, and the depth at which embryos are exposed. Studies of several marine fish species have documented increased mortality of embryos that were exposed to air gun blasts within 1 m of the source (Kostyuchenko 1973; Booman et al. 1996; Payne 2004). We hypothesized that exposure to a seismic air gun has the potential to reduce survival in lake trout embryos within 3 m of the sound source. Developing salmonid embryos undergo a "sensitive" period between 48 h postfertilization and eye-up, during which time hatchery personnel refrain from handling the embryos (Piper et al. 1982). In lake trout, this time corresponds to the final stages of epiboly (Fitzsimmons 1994) as the germ ring closes and a neural plate forms (Balon 1980). We hypothesized that sound from a seismic air gun has the greatest potential to increase mortality during the sensitive period, with efficacy decreasing as development progresses. The effects of a seismic air gun discharge on lake trout embryos may also vary with depth, as the varying density

of water (due to temperature gradient) and pressure conditions could influence the transmission of sound waves or the sensitivity of embryos. We hypothesized that increased pressure and water density may increase the sensitivity of lake trout embryos to a discharge from a seismic air gun.

METHODS

A $2 \times 2 \times 2$ factorial design was implemented to evaluate the effects of a 655.5-cm³ (40-in³) seismic air gun on lake trout embryos at several stages of development. Treatments consisted of two operation levels (exposure and mock exposure [i.e., control]) at two distances from the air gun (0.1 and 2.7 m) and at two depths (5 and 15 m) that were representative of lake trout spawning depths in lakes of the western USA (Cox 2010; Dux et al. 2011). Three replicates of each treatment were conducted. The experiment was repeated for each developmental stage by using the factorial design.

Embryos were contained in boxes made of 2-mm square mesh (low-density polyethylene [LDPE]). Box dimensions (length \times width \times height) were 7.62 \times 7.62 \times 2.54 cm. Fifty embryos were assigned to numbered egg boxes for each replicate within each experiment. Treatments were then randomly assigned to numbered egg boxes.

We assumed that the open meshes used to contain embryos did not affect sound pressure inside the containers. Technical difficulties prevented us from measuring sound pressure inside the egg boxes. After initial sound pressure measurements were made, the hydrophone malfunctioned and would not measure the acoustic signal in subsequent trials with the container materials. In a similar experiment, sound pressure measurements inside solid-walled LDPE cubitainers did not differ from simultaneous measurements taken outside the containers (Pearson et al. 1994).

Experiments were conducted by suspending egg boxes and the air gun from an aluminum bar that was attached to an improvised trawling frame on an 8.8-m vessel. Hydraulic winches on either side of the vessel were used to raise and lower the apparatus to the experimental depths. Winch cables were marked to ensure consistent depth and position of the apparatus (Figure 1). An egg box was suspended at 0.1 and 2.7 m on either side of the air gun; thus, one replicate at each distance was conducted per operation \times depth treatment. The 655.5-cm³ sleeve gun (Ion Geophysical) was pressurized to 13,789.5 kPa (2,000 lb/in²) and was discharged once for exposure replicates. Mock exposure (i.e., control) replicates were treated identically except that the air gun was not pressurized or discharged. The order of operation \times depth treatments was randomized to maintain temporal independence during each experiment. The boat was anchored in 25–30 m of water in each experiment.

Lake trout eggs were collected during annual lake trout removal netting at spawning locations in Lake Pend Oreille, Idaho. Eggs were fertilized by Idaho Department of Fish and Game hatchery personnel on 7 October 2010 by using gametes from several female and male lake trout. Fertilized embryos were water-hardened, transported to Priest Lake, Idaho, and

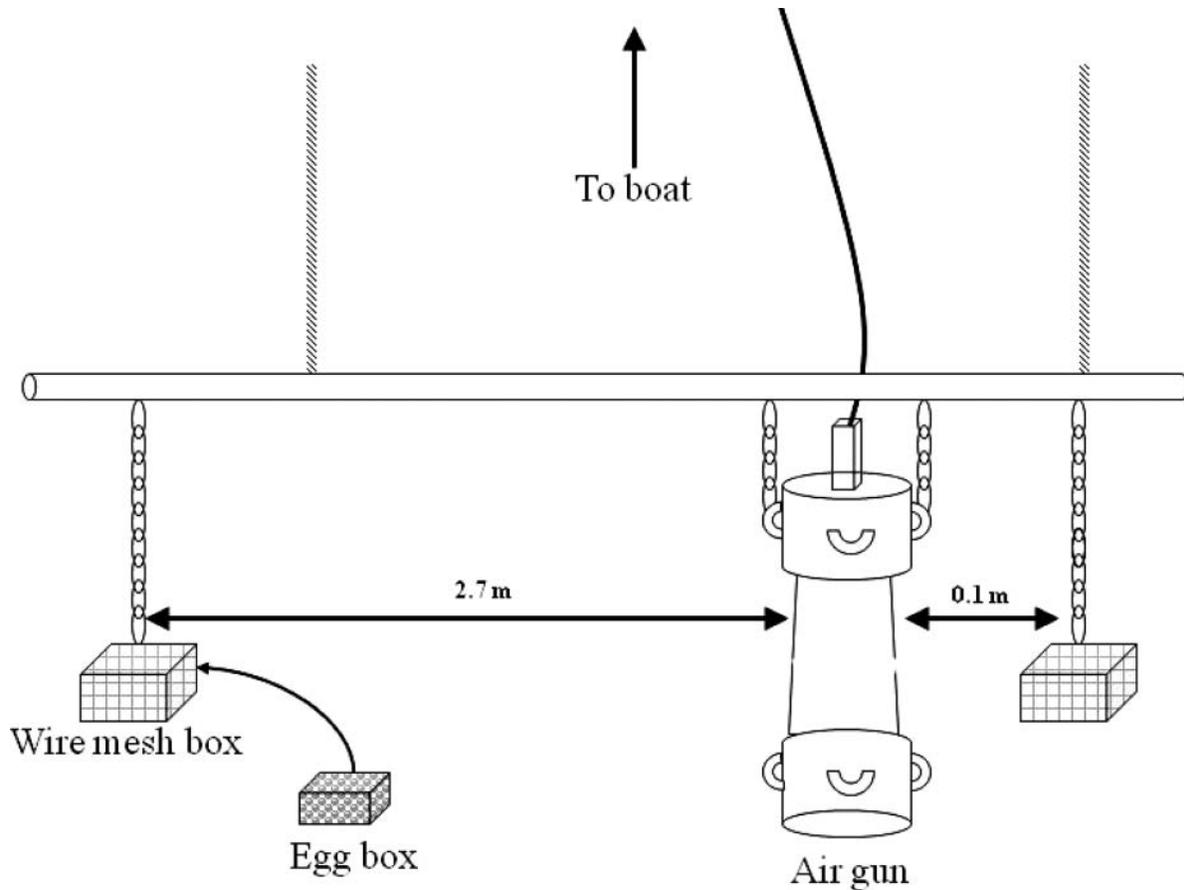


FIGURE 1. Diagram of the apparatus used to expose lake trout embryos to a 655-cm³ (40-in³) air gun in Priest Lake, Idaho, during autumn 2010.

loaded into numbered egg boxes within 24 h. Egg boxes were held within a wire-mesh incubation box that was suspended at 6.6 m until treatment. One incubation box was treated at 5, 10, 15, and 20 d postfertilization, which corresponded to 74, 156, 207, and 267 daily temperature units in degrees Celsius (TU°C). The experiments with fish at 74 and 156 TU°C represented two “sensitive” stages in development. Mortality to eye-up was measured as the proportion of dead embryos in each replicate after the 267-TU°C experiment. Live eyed embryos were transported to the wet laboratory at the University of Idaho on 1 November 2010 for monitoring of survival to hatch. The polyethylene mesh boxes containing the embryos were placed into Heath trays, and embryos were incubated at 9.5°C until all had hatched or died.

Air gun pressure signatures were measured with a calibrated hydrophone (SmartPhone v1.02; AG Geophysical) and recorded at 1-ms intervals to a computer via an RTS Hotshot control box (Real-Time Systems, Inc.). A minimum of four signatures were recorded at each treatment distance and depth. To describe the pressure signature of each treatment level, the mean of the signatures was calculated for all depth \times distance combinations. Peak sound pressure level (SPL_{0-Peak}; dB referenced to [re] 1 μ Pa) for each treatment level was calculated from the mean pressure signature (bar) as $20 \cdot \log_{10}(\text{bar} \cdot 10^{11}/1)$ (Gausland 2000).

Mortality to eye-up in treatment and control groups of lake trout embryos was compared by using box plots for each developmental stage. Chronic mortality effects in embryos were examined by using logistic regression (Hosmer and Lemeshow 2000). Specifically, logistic regression was used to compare mortality rates in treatment and control groups during incubation at the wet laboratory. The day on which all embryos were alive (i.e., at fertilization) was included as the first point in time so that data for all groups originated at the same point. The proportion of dead embryos was treated as the dependent variable, time was treated as a continuous independent variable, and treatment was used as a categorical independent variable. A full time \times treatment interaction model (i.e., different slopes and intercepts for all treatment groups) was compared with an additive model (i.e., different intercepts and a common slope) and a model that included a common slope and common intercept (date effect only). Models were ranked by using Akaike’s information criterion corrected for small sample size (AIC_c; Burnham and Anderson 2002). The model with the lowest AIC_c value was considered the best-performing model. Models were considered to be supported by the data equally if the difference in AIC_c relative to that of the top model (i.e., ΔAIC_c) was less than 2 (Burnham and Anderson 2002).

TABLE 1. Mean (SD in parentheses) peak sound pressure levels (SPL_{0-Peak}) of air gun discharge recorded for depth × distance treatments in lake trout embryo experiments (Priest Lake, Idaho; autumn 2010).

| Distance (m) | Depth (m) | SPL _{0-Peak} (dB re 1 μPa) |
|--------------|-----------|-------------------------------------|
| 0.1 | 5 | 232 (0.40) |
| 2.7 | 5 | 209 (0.72) |
| 0.1 | 15 | 225 (1.46) |
| 2.7 | 15 | 207 (0.93) |

RESULTS

Peak SPL of the air gun discharge varied from 207 to 232 dB re 1 μPa among the treatment depths and distances (Table 1). At both depths, SPL_{0-Peak} was highest for treatments at 0.1 m from the air gun. At both distances, SPL_{0-Peak} decreased with greater depth. Although the air gun was pressurized to 13,789.5 kPa (2,000 lb/in²) for each discharge, peak pressure varied slightly from shot to shot. Variation in SPL_{0-Peak} measurements was greatest at 0.1 m from the air gun at the 15-m depth.

Mortality of lake trout embryos exposed to the air gun at 74 TU°C (5 d postfertilization) was higher than that of control groups for the 0.1-m treatment at both depths (Figure 2). All f5-d-old embryos exposed at 0.1 m appeared to be dead, as they turned white before being returned to the incubation boxes on the day of treatments; none of these embryos survived to eye-up. Mortality of lake trout embryos at 74 TU°C treated at a distance of 2.7 m did not appear to differ from that of controls at either depth.

For lake trout embryos at 156 TU°C, exposure to the air gun at 0.1-m distance appeared to cause higher mortality than that of control groups, with the exception of one treatment group at the 15-m depth (Figure 2). One replicate was lost at the 0.1-m distance × 5-m depth treatment (the air gun blast ruptured the mesh egg box). Mortality of embryos treated at the 2.7-m distance did not appear to differ from mortality of controls at either depth.

At 207 TU°C, mortality of embryos treated at a distance of 0.1 m appeared to be higher than that of controls at 15-m depth but not at 5-m depth (Figure 2). Variation in mortality was greatest for embryos that were exposed to the air gun at the 0.1-m distance and 5-m depth. Treatments at a distance of 2.7 m did not appear to produce mortality differing from that of controls at either depth.

Embryos reached eye-up by 20 d postfertilization (267 TU°C). At this stage, mortality to eye-up in the 0.1-m treatment groups was higher than mortality of controls at 15-m depth but not at 5-m depth (Figure 2). Mortality did not appear to be different between treatment and control embryos (267 TU°C) at the 2.7-m distance for either depth.

Within each developmental stage, mortality in control groups at the two treatment depths appeared to be relatively consistent. However, mortality to eye-up in control groups

TABLE 2. Model selection results comparing logistic models of mortality through time for lake trout embryos that were incubated at the University of Idaho wet lab in November 2010 after exposure to a seismic air gun (AIC_c = Akaike's information criterion corrected for small sample size; ΔAIC_c = difference in AIC_c between the given model and the best-performing model).

| Model | AIC _c | ΔAIC _c | AIC _c weight |
|--|------------------|-------------------|-------------------------|
| Unique slope and unique intercept for each group | 6,929.7 | 0.0 | 1 |
| Common slope but unique intercept for each group | 6,975.4 | 45.7 | 0 |
| Common slope and common intercept | 8,418.34 | 1,488.7 | 0 |

varied from 22% to 92% across all developmental stages and depths (Figure 3). For the four lake trout developmental stages, exposure to the air gun at a distance of 0.1 m resulted in acute mortality that was up to 60% greater than the mortality among controls (Figure 3, top panel). Mortality in the 0.1-m treatments was at least 20% greater than that of corresponding controls; exceptions were embryos at 207 and 267 TU°C receiving the 5-m depth treatment. For treatments at 0.1 m from the air gun, exposure at 15-m depth had a greater effect size (i.e., difference of each treatment replicate from the average mortality of controls at the corresponding depth and developmental stage) on the 207- and 267-TU°C developmental stages than did shallow (5-m) exposure (Figure 3). The effect of air gun discharge at a distance of 2.7 m appeared to be negligible across developmental stages and depths (Figure 3, bottom panel).

There was little evidence of chronic mortality in embryos after exposure to the air gun. Although mortality during incubation in the wet laboratory was best described with unique logistic regression parameters for each treatment (Table 2), confidence intervals on the estimated slope parameters overlapped among all treatments.

DISCUSSION

This study provides evidence that a seismic air gun is capable of increasing mortality in lake trout embryos when they are exposed to the blast in close proximity (0.1 m). Few studies have reported adverse effects of air gun exposure on embryonic fish (Dalen and Knutsen 1986; Payne 2004; Payne et al. 2009); however, two studies indicated that exposure to a seismic air gun within 1 m increased mortality in fish embryos (Kostyuchenko 1973; Booman et al. 1996). Survival of various commercial fish species from the Black Sea when exposed to a 5-L air gun at 0.5 m was 17% lower than the survival of controls (SPL was not reported; Kostyuchenko 1973). Mortality in pollock *Pollachius virens* at an early stage of embryonic development was higher than that of controls for treatments exposed to a seismic air gun at 0.75-m distance (SPL = 242 dB re 1 μPa; Booman et al. 1996). In the present study, the air gun blast at the 2.7-m distance

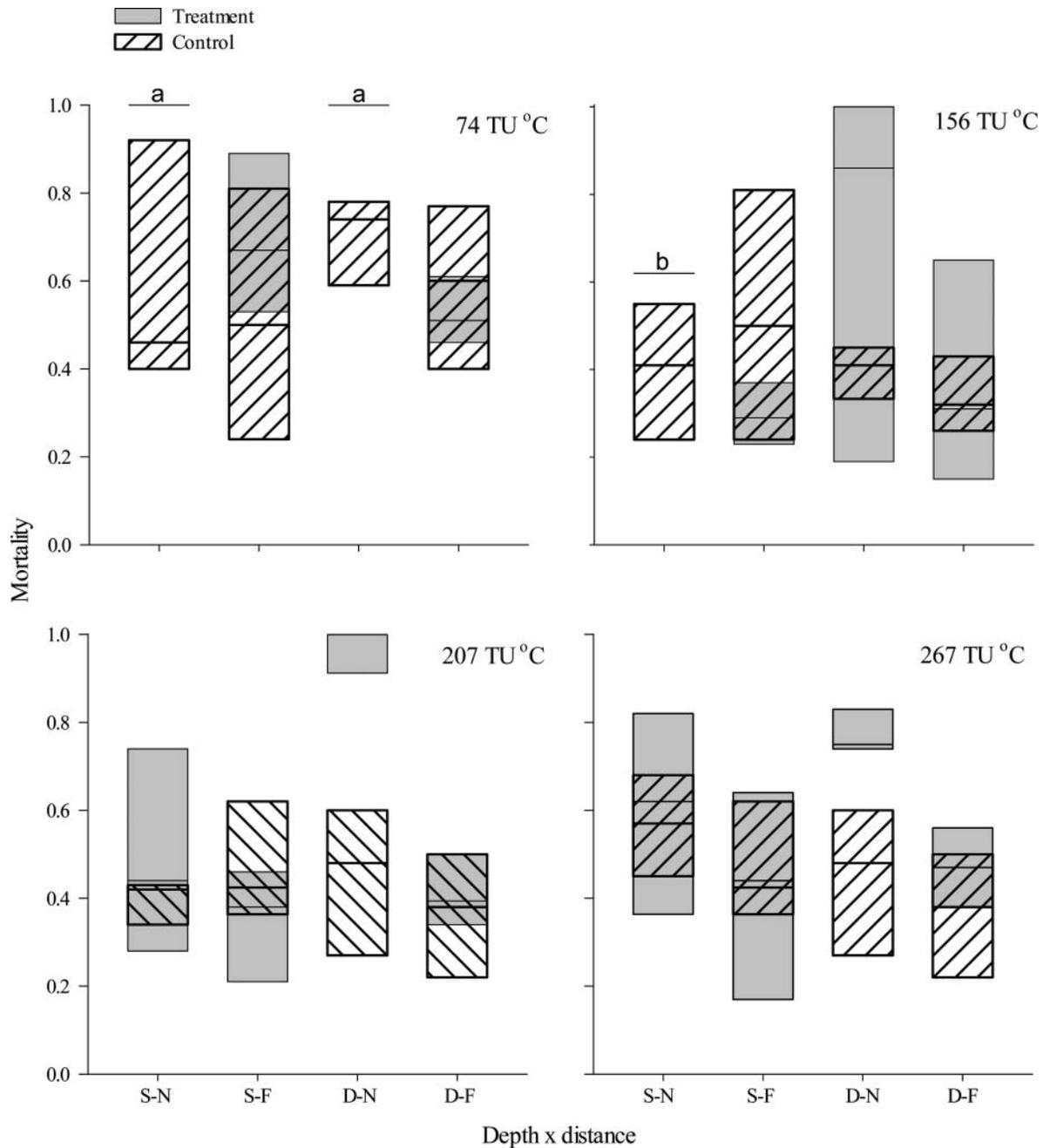


FIGURE 2. Box plots comparing mortality to eye-up for lake trout embryos in air gun experiments conducted in Priest Lake, Idaho, during autumn 2010. Labels on the x-axis represent depth \times distance treatments (S = shallow, 5-m depth; D = deep, 15-m depth; N = near, 0.1-m distance; F = far, 2.7-m distance). The corresponding developmental stage (daily temperature units [TU $^{\circ}$ C]) at the time of treatment is displayed in the upper right corner of each panel. The solid line in the middle of each box represents the median of three replicates at each treatment level. Upper and lower boundaries of the box represent the range of the data. Thin lines (labeled with an "a") for S-N at 74 TU $^{\circ}$ C represent three treatment replicates with 100% mortality in each. The thin line (labeled with a "b") represents the mean of two observations, as one treatment replicate was lost.

(207–209 dB re 1 μ Pa) did not affect lake trout embryos, regardless of developmental stage. Thus, the effective range for reducing lake trout embryo survival with a single 655-cm 3 air gun is probably between 0.1 and 2.7 m (232–207 dB re 1 μ Pa). Embryos in this study were only exposed to one discharge of the

air gun; thus, multiple air gun blasts may be more detrimental to sensitive embryos.

Data from this experiment support the hypothesis that developmental stage can influence the efficacy of the air gun in causing mortality of salmonid embryos. Lake trout embryos

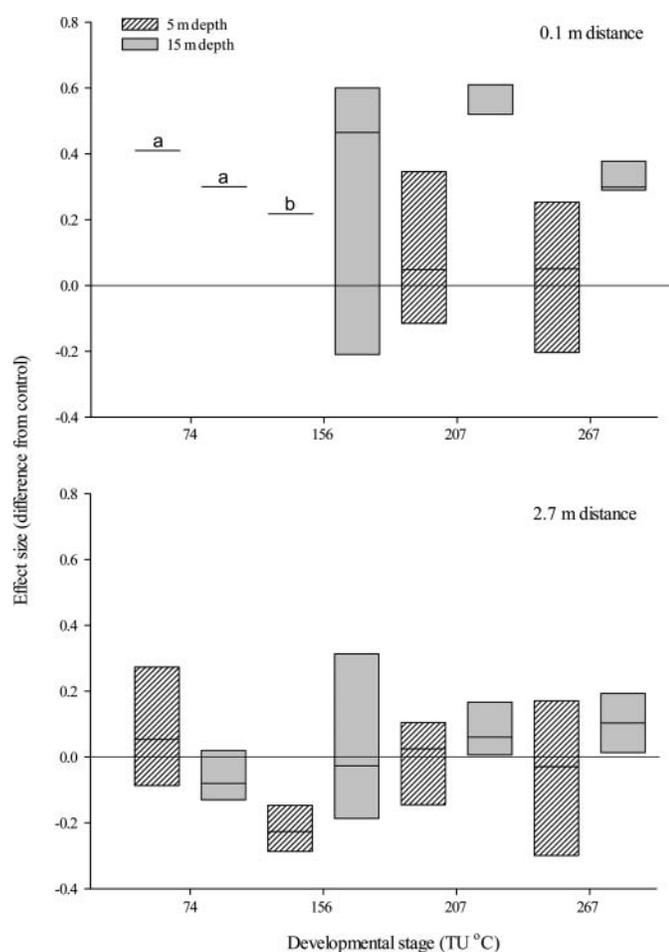


FIGURE 3. Box plots comparing effect sizes of the 0.1-m (upper panel) and 2.7-m (lower panel) distance treatments at two depths across developmental stages (daily temperature units [TU[°]C]) for lake trout embryos exposed to a seismic air gun in Priest Lake, Idaho, during autumn 2010. Effect size was calculated as the difference of each treatment replicate from the average mortality of controls at the corresponding depth and developmental stage. In the top panel, thin lines (labeled with an “a”) at 74 TU[°]C represent three replicates with 100% mortality in each. The thin line (labeled with a “b”) represents the mean of two observations, as one treatment replicate was lost.

are known to be sensitive to physical shock during early ontogeny (Fitzsimmons 1994). In a hatchery setting, survival of lake trout embryos was reduced by 20% via manual shaking of the embryos at 5–8 d postfertilization (Fitzsimmons 1994). In our study, treatments of lake trout at 74 TU[°]C (5 d postfertilization) resulted in 100% mortality. Use of seismic technology as a control method may be most effective if it is focused on embryos during early development. Detailed knowledge of the timing of spawning will be necessary to ensure that the embryos are treated during the sensitive stage.

Data from this experiment also support the hypothesis that the depth at which embryos are exposed can influence the efficacy of the air gun. Effect sizes of the 0.1-m treatments remained high at 15-m depth for lake trout embryos at the advanced stages

of development (i.e., 207 and 267 TU[°]C), whereas effect sizes of shallow (5-m) treatments at these developmental stages appeared to be negligible. Thus, a depth × developmental stage interaction may have affected mortality in treatment groups at the 0.1-m distance. The measured air gun signatures illustrated that SPL_{0-Peak} was reduced at 15-m depth. Elevated ambient pressure at 15-m depth would have reduced the hydrostatic gradient between the air gun and the environment, thus reducing the explosive potential of the pressurized air gun. At some depth, the reduced pressure gradient would be likely to reduce the SPL_{0-Peak} below the lethal level. Although SPL_{0-Peak} was reduced at 15-m depth, we surmise that embryonic salmonids may be more sensitive to an air gun blast with increased depth. Increased pressure and density of water (due to thermal stratification) at 15 m would allow for more rapid attenuation of the blast, and the sound wave would therefore stretch and compress the embryos more rapidly. The relatively consistent mortality between controls at 5- and 15-m depth within each developmental stage suggest that effects of changing pressure and temperature in the absence of an air gun blast had little effect on background mortality within the range of spawning depths observed in natural lake trout populations (5–15 m).

Relatively high ambient water temperatures in Priest Lake may have contributed to the relatively high levels of background mortality in two ways. Temperatures at the incubation site were above the upper limit for spawning in natural lake trout populations (13°C) for 12 d postfertilization (Sly and Evans 1996). Embryos held at this temperature would have developed at an increased rate, which can lead to physical abnormalities (Ojan-guren et al. 1999). The elevated temperatures during incubation also probably contributed to an observed high incidence of fungal infection while embryos incubated in Priest Lake. Initially, we intended to compare survival to hatch between treatment and control embryos, but only one embryo survived to hatch in the wet laboratory. In future studies, delaying the experiments until surface temperatures decline in the autumn (i.e., to more closely approximate natural lake trout spawning conditions) would help to reduce incidence of fungal infection.

Although background mortality was high in these experiments, it was relatively consistent within developmental stages. In a hatchery setting, mortality rates as low as 10% are possible (e.g., Fitzsimmons 1994). In our study, dead embryos were not removed from egg boxes until the embryos reached eye-up; this was done to minimize handling during the sensitive period. Fungal infection was responsible for much of the background mortality in treatment and control groups. Ideally, future experiments should be conducted in a laboratory setting or an on-site hatchery facility, where treatment options are available to control fungal outbreaks. Minimizing background mortality in future investigations of seismic air gun technology would help to ensure detection of treatment effects.

These data indicate that a relatively small seismic air gun can increase mortality in lake trout embryos; however, much work remains to determine whether this technology can be used

as tool for nonnative species control. Mortality of embryos exposed in close proximity (i.e., 0.1 m) to the air gun appeared to be greater than the mortality of controls. To be a practical tool for conservation, air gun technology must be capable of inducing mortality at a greater distance, as lake trout embryos may settle as deep as 1 m into the interstices of cobble and boulder substrates from 8 cm to 3 m (Marsden et al. 1995). Use of larger air guns can produce higher SPL_{0-Peak} at greater distances and depths and should be examined in future studies. Quantifying the lethal range (or SPL) of a seismic air gun blast with greater precision (i.e., treating the distance or SPL as a continuous variable) and understanding the influence of hard substrates (e.g., reflection) on air gun lethality are immediate research needs for determining whether this is a feasible technique for controlling invasive salmonids. Large lakes may contain several square kilometers of spawning habitat for lake trout (Marsden et al. 1995). Realistically, employing an array of larger air guns may be the only means of covering the extensive spawning areas that are common in large lakes containing nonnative lake trout.

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