

**USE OF SEISMIC AIR GUNS TO REDUCE
SURVIVAL OF SALMONID EGGS AND
EMBRYOS: A PILOT STUDY**

Completion Report

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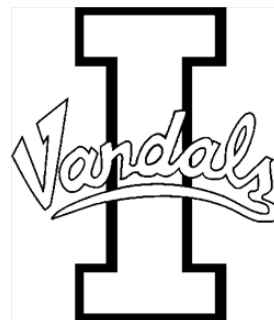


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ABSTRACT

The detrimental impacts of nonnative lake trout *Salvelinus namaycush* to ecosystems in the western US have prompted natural resource management agencies in several states to implement lake trout suppression programs. These programs rely on mechanical removal methods (i.e., gill nets, trap nets, and angling) to capture subadult and adult lake trout. Targeting embryonic lake trout at spawning locations could be an effective strategy to supplement mechanical removal techniques utilized by lake trout suppression programs. We conducted a study to explore the potential for using high-intensity sound to reduce survival in lake trout embryos with a relatively small (655.5 cm³, 40 in³) seismic air gun. Lake trout embryos at multiple stages of development (fertilization to eye-up) were exposed to a single discharge of the seismic air gun at two depths (5 m and 15 m) and two distances from the air gun (0.1 m and 2.7 m). Experiments were also conducted on rainbow trout *Oncorhynchus mykiss* and kokanee salmon *Oncorhynchus nerka* at advanced stages of development (i.e., eye-up to hatch). Mortality in lake trout embryos treated at 0.1 m from the air gun appeared higher than control groups at 74 and 156 daily temperature units in degrees Celsius (TU°C) at both depths. Mortality in lake trout embryos treated at 0.1 m from the air gun at 207 and 267 TU°C appeared higher than controls at the 15 m depth. Mortality at the 2.7 m distance did not appear to differ from controls at any developmental stage or either depth. Although the relatively small air gun employed in this study is not practical for large-scale suppression efforts, these data indicate seismic air guns have potential as an alternative tool for controlling nonnative lake trout. We conclude further investigation into this technology is warranted.

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INTRODUCTION

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 US, 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 bouvierii* 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). The effects of nonnative lake trout extend beyond the fish community in ecosystems of the western US. 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 where they have caused declines in populations of migratory fish species used by avian and mammalian predators during spawning runs (Spencer et al. 1991; Koel et al. 2005).

The detrimental impacts of nonnative lake trout in the western US 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. Elasticity analyses of matrix population models suggest that lake trout population growth is highly sensitive to changes in survival from age 0 to age 1 (Cox 2010). Thus, targeting embryonic lake trout with alternative control methods could be an effective supplement to current mechanical removal techniques.

Using 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). As demand and exploration (i.e., seismic surveys) for offshore petroleum has increased, there has been increased concern over 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 salmonid embryos have yet to be studied.

The objective of this study was to explore the potential for using a relatively small seismic air gun, practical for use by a small crew of agency personnel, as a means of reducing survival in lake trout embryos. The effect of a seismic discharge on embryonic lake trout likely depends on several factors, including sound intensity, physiological characteristics related to the developmental stage of the embryo, and the depth at which embryos are exposed. Increased mortality in fish embryos has been documented in several marine species exposed to air gun blasts within 1 m of the source (Kostyuchenko 1973; Booman et al. 1996; Payne 2004). We hypothesize exposure to a seismic air gun has the potential to reduce survival in lake trout embryos within three meters of the sound source. Developing salmonid embryos undergo a "sensitive" period from 48 h post-fertilization to eye-up, during which time hatchery personnel refrain from handling 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 hypothesize 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 hypothesize that increased

pressure and water density may increase the sensitivity of salmonid embryos to discharge from a seismic air gun.

METHODS

Experimental design

A 2x2x2 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 m and 2.7 m) and two depths (5 m and 15 m). Three replicates of each treatment were conducted (Table 1). The experiment was repeated for each developmental stage using the factorial design.

Embryos were contained in boxes made of low-density polyethylene (LDPE) mesh (2 mm square). Boxes were 7.62 cm long by 7.62 cm wide by 2.54 cm high. Fine mesh fiberglass window screen was fixed to egg boxes to prevent sac-fry from escaping in experiments on embryos near hatching. Fifty embryos were assigned to numbered egg boxes for each replicate in each experiment. Treatments were then randomly assigned to numbered egg boxes.

We assumed open meshes used to contain embryos did not affect sound pressure inside the containers. In a similar experiment, sound pressure measurements inside LDPE Cubitainers® did not differ from simultaneous measurements outside the container (Pearson et al. 1994). Technical difficulties with the hydrophone following initial sound pressure measurements prevented us from measuring sound pressure inside the container materials.

Experiments were conducted by suspending egg boxes and the air gun from an aluminum bar 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 meters on either side of the air gun, thus one replicate at each distance was conducted per operationxdepth treatment. The air gun was pressurized to 13,789.5 kPa (2,000 psi) and 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 operationxdepth treatments was randomized to maintain temporal independence during each experiment. The boat was anchored in 25-30 m in each experiment.

Embryos

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 (IDFG) hatchery personnel on 7 October 2010 using gametes from several female and male lake trout. Eggs were transported to Priest Lake and loaded into numbered egg boxes. Egg boxes were placed in a wire mesh incubation box for the corresponding developmental stage experiment. Embryos were treated at 74, 156, 207, and 267 TU°C (daily temperature units in °C). The 74 and 156 TU°C experiments represented two “sensitive” stages in development. Mortality to eye-up was measured as the proportion of dead embryos in each replicate following the 267 TU°C experiment. Live embryos were transported to the University of Idaho (UI) wet lab on 1 November 2010 to monitor survival to hatch. Embryos were incubated in

the polyethylene mesh boxes contained in Heath trays at 9.5°C until all embryos had hatched or died.

Although we were primarily interested in studying the effects of the air gun on lake trout embryos, we sought to maximize our opportunity to collect information with the air gun equipment. Therefore, we conducted experiments on two additional salmonid species (i.e., rainbow trout *Oncorhynchus mykiss* and kokanee *Oncorhynchus nerka*). Rainbow trout eggs were obtained from Troutlodge Inc. (Sumner, WA) on 17 September 2010 and incubated at the IDFG Sandpoint Fish Hatchery until embryos were treated on 29 September 2010 at approximately 332 TU°C. A sample of eyed kokanee eggs were obtained from the IDFG Cabinet Gorge Fish Hatchery on 13 October 2010, acclimated in Priest Lake overnight, and treated at 442 TU°C on 14 October 2010. Acute mortality was measured for rainbow trout and kokanee experiments as the proportion of dead embryos in each replicate after incubating for 72 h following treatment.

Sound measurements

Air gun pressure signatures were measured with a calibrated hydrophone (A-G Geophysical Products, Inc. SmartPhone v1.02) and recorded at 1 ms intervals to a computer via a 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 and distance combinations. Peak sound pressure for each treatment level (SPL_{0-Peak} ; dB re 1 μ Pa) was calculated from the mean pressure signature in bar as $20 \cdot \log_{10} (\text{bar} \cdot 10^{11}/1)$ (Gausland et al. 2000).

Data analysis

Mortality to eye-up in treatment and control groups of lake trout embryos were compared using box plots within each developmental stage. Box plots of mortality data (72 h post-exposure) were also constructed to compare treatments and controls for rainbow trout and kokanee experiments. Chronic mortality effects in lake trout embryos were examined 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 UI wet lab. The day at which all eggs were alive (i.e., fertilization) was included as the first point in time so that data for all groups originated at the same point. The proportion of dead to total embryos was treated as the dependent variable with time as a continuous independent variable and treatment as a categorical independent variable. A full-time treatment interaction model (i.e., different slopes and intercepts for all treatment groups) was compared to an additive model (i.e., different intercepts, common slope) and a common slope-intercept model (date effect only). Models were ranked by AIC_c to account for small sample sizes (Burnham and Anderson 2002). The model with the lowest AIC_c was considered the top model. Models were considered to be supported by the data equally if the difference in AIC_c from the top model (i.e., ΔAIC_c) was <2 (Burnham and Anderson 2002).

RESULTS

Peak sound pressure of the 655.5 cm³ (40 in³) gun discharge varied from 207 to 232 dB re 1 μ Pa among the treatment depths and distances. At both depths, peak sound pressure was highest for treatments at 0.1 m from the air gun (Figure 2). Peak sound pressure decreased at

both distances with increased depth. Although the air gun was pressurized to 13,789.5 kPa (2,000 psi) for each discharge, peak pressure varied slightly from shot to shot. Variation in peak sound pressure measurements was greatest at 0.1 m from the air gun at the 15 m depth.

Exposing rainbow trout and kokanee embryos to the seismic air gun did not appear to result in increased mortality at either distance or depth when compared to controls (Figure 3). However, variation in mortality in treatment groups was greater than in controls at both depths and distances for rainbow trout. Mortality in kokanee embryos was low in both treatment and control groups (0-5%), but varied more within the 0.1 m treatment groups. Overall, mortality in treatments was not consistently greater than controls at either depth or distance for rainbow trout and kokanee embryos at advanced developmental stages (i.e., late eye-up to hatch).

Mortality in lake trout embryos exposed to the air gun at 74 TU°C (five days old) was higher than control groups for the 0.1 m treatment at both depths (Figure 4, panel A). All five-day-old embryos exposed at 0.1 m were dead and turned white before they were returned to the incubation boxes on the day of treatments; none survived to eye-up. However, mortality of lake trout embryos at 74 TU°C treated at 2.7 m did not appear different from controls at either depth.

Lake trout embryos at 156 TU°C exposed to the air gun at 0.1 m exhibited higher mortality than control groups, with the exception of one treatment group at the 15 m depth (Figure 4, panel B). One replicate was lost at the 0.1 m distance x 5 m depth treatment, as the air gun blast ruptured the mesh egg box. Mortality in embryos treated at the 2.7 m distance did not appear different from controls at either depth.

Mortality in lake trout at 207 TU°C treated at 0.1 m appeared higher than controls at 15 m depth but not at the 5 m depth (Figure 4, panel C). Variation in mortality was greatest for the 0.1 m distance treatment at 5 m depth. Treatments at 2.7 m did not appear to differ from controls at either depth.

Lake trout embryos reached eye-up by 20 days post-fertilization (267 TU°C). At this stage, mortality to eye-up in 0.1 m treatment groups was higher than controls at 15 m depth but not at 5 m (Figure 4, panel D). Mortality did not appear to be different between treatments and controls at 2.7 m from the air gun at either depth at 267 TU°C.

Control groups at the two treatment depths appeared similar within each developmental stage (Figure 5). Mortality in the controls was highest at the 74 TU°C developmental stages but was more consistent in later developmental stages. Mortality to eye-up in lake trout control groups varied from 22% to 92% for all developmental stages and depths.

Exposure to the air gun at 0.1 m resulted in acute mortality up to 60% greater than controls among the four lake trout developmental stages (Figure 6, top panel). Mortality was at least 20% greater than corresponding controls, with the exception of the 5 m depth treatments at 207 and 267 TU°C. Treatments at 0.1 m from the air gun at 15 m depth had large effect sizes in the latter developmental stages (207 and 267 TU°C) relative to shallow treatments. The effect of the air gun discharge at 2.7 m was negligible across developmental stages and depths (Figure 6, bottom panel).

There was little evidence of chronic mortality following exposure to the air gun. Lake trout mortality data during incubation in the UI wet lab was best described with unique logistic regression parameters for each treatment (Table 2). There was little support for modeling mortality through time with common slope or intercept parameters for the different treatment

groups, as ΔAIC_c was greater than 10 for these models relative to top model. However, slope parameters were not different for the different treatment and control groups (Figure 7). Models of mortality through time for both treatment and control groups were similar, with the exception of one 0.1 m treatment at 15 m for embryos at 207 TU°C in which few eggs survived to the hatchery (Figure 8).

DISCUSSION

This study provides evidence that a seismic air gun is capable of increasing mortality in salmonid embryos exposed within 0.1 m of the blast. Few studies have reported increased mortality in embryonic fish exposed to air guns (Dalen and Knutsen 1986; Payne 2004; Payne et al. 2009); however, two studies have shown 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 was 17% lower than controls when exposed to a 5 L air gun at 0.5 m (SPL not reported; Kostyuchenko 1973). Mortality in saithe *Pollachius virens* eggs at an early stage of development was higher than controls for treatments exposed to a seismic air gun at 0.75 m distance (SPL = 242 dB re 1 μ Pa; Booman et al. 1996). In this study, the effect of the blast at 2.7 m distance (207-209 dB re 1 μ Pa) was negligible in all species and developmental stages. Thus, the effective range for reducing survival in lake trout embryos using a single 655 cm³ (40 in³ gun) likely occurs between 0.1-2.7 m (232-207 dB re 1 μ Pa).

Mortality to eye-up in lake trout data support the hypothesis that developmental stage can influence the efficacy of the air gun on salmonid embryos. Lake trout embryos are known to be sensitive to physical shock during early ontogeny (Fitzsimmons 1994). Survival in a hatchery setting has been reduced 20% by manually shaking lake trout embryos 5-8 days post-fertilization (Fitzsimmons 1994). In this study, treatments of lake trout at 74 TU°C resulted in 100% mortality. Rainbow trout and kokanee embryos treated at advanced stages (i.e., late eye-up to hatch) of development did not have consistently higher mortality than controls for either depth. Using seismic technology as a control method may be most effective if focused on embryos during early development. Detailed knowledge of the timing of lake trout spawning will be necessary to ensure eggs are treated during the sensitive stage.

Data from the lake trout experiments also support the hypothesis that the depth at which embryos are exposed can influence the efficacy of the air gun. Effect sizes of 0.1 m treatments remained high at 15 m depth in lake trout embryos at the advanced stages of development (i.e., 207,267 TU°C), where effect sizes in shallow treatments were negligible. Thus, a depth \times developmental stage interaction may have affected mortality in treatment groups at the 0.1 m distance. The measured air gun signatures illustrated that depth influenced the peak pressure output of the air gun blast. Peak sound pressures were lower at 15 m depth due to a reduced pressure gradient across the air gun chamber and the water column. We surmise that embryonic salmonids may be more sensitive with increasing depth because increased ambient pressure and density of water (due to thermal stratification) would allow for faster transmission of sound. The relatively consistent mortality between 5 m and 15 m depth controls 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.

Minimizing background mortality should be a priority in future investigations to ensure detection of treatment effects. Background mortality was relatively low in rainbow trout and kokanee experiments, thus we should have been able to detect an effect on treatment groups

72 h following the experiments. Although background mortality was high in lake trout 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 this study, dead embryos were not removed from egg boxes until eggs reached eye-up, with the intention of minimizing handling during the sensitive period. Fungal infection was responsible for much of the background mortality in treatments and controls. Ideally, future experiments should be conducted in a laboratory setting or in an onsite hatchery facility to control fungal outbreaks. With lower background mortality rates and continuous monitoring of mortality, detecting chronic mortality effects in treatment groups would be more likely with logistic regression models.

Relatively high temperatures in Priest Lake may have contributed to background mortality in lake trout embryos in two ways. Temperatures at the incubation site were above the upper limit for spawning in natural lake trout populations (13°C) for 12 days post-fertilization (Sly and Evans 1996). Embryos would have developed at an increased rate at this temperature, which can lead to physical abnormalities in embryos (Ojanguren et al. 1999). The elevated temperatures during incubation also likely contributed to the high incidence of fungal infection while embryos incubated in Priest Lake. Initially, we aimed to compare survival to hatch out between treatment and controls, but only one embryo survived to hatch in the wet lab. Delaying experiments until surface temperatures have declined may reduce incidence of fungal infection and physical abnormalities in a field setting.

These data indicate a relatively small seismic air gun can increase mortality in lake trout embryos; however, much work remains to determine if this technology can be used as tool for suppression. Mortality appeared greater than controls for embryos in close proximity (i.e., 0.1 m) to the air gun. Inducing mortality at a greater distance is likely necessary at lake trout spawning sites, as lake trout eggs can occur as deep as 1 m into the interstices of rock substrates (Marsden et al. 1995). Quantifying the effective range of seismic air guns in more detail and understanding the influence of rock substrates on embryo mortality are necessary to determine if air guns are a feasible control tool. If the sound from an air gun cannot reach embryos with lethal force in natural substrates, this technology may not be practical as a control technique. Further, larger air guns than used in this study are available and should be tested in future experiments. Larger air guns (or an array of air guns) can produce higher sound pressure at greater distances. Large lakes may contain several square kilometers of spawning habitat for lake trout (Marsden et al. 1995). Employing an array of larger air guns may be the only realistic means of covering extensive spawning areas common in the large western lakes containing nonnative lake trout. Embryos in this study were only exposed to one discharge of the air gun. If this technology were to be applied as a control technique, the air gun would most likely be discharged continuously over spawning areas. Thus, information on the effects of multiple air gun discharges is also necessary to determine if this technology is a feasible tool for controlling nonnative lake trout.

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TABLES

Table 1. Factorial design for experiments on lake trout embryos with 655 cm³ (40 in³) seismic air gun conducted in Priest Lake, Idaho, autumn 2010.

Air gun discharge(Treatment)			Mock air gun discharge(Control)		
Depth	Distance from air gun		Depth	Distance from air gun	
	0.1 m	2.7 m		0.1 m	2.7 m
5 m	3 reps	3 reps	5 m	3 reps	3 reps
15 m	3 reps	3 reps	15 m	3 reps	3 reps

Table 2. Model selection results comparing logistic models of mortality through time in lake trout embryos incubated in the University of Idaho wet lab, November 2010.

Model	AICc	ΔAICc	AICc weight
Slope and intercept for each group	6929.7	0.0	1
Common slope, intercept for each group	6975.4	45.7	0
Common slope and intercept	8418.34	1488.7	0

FIGURES

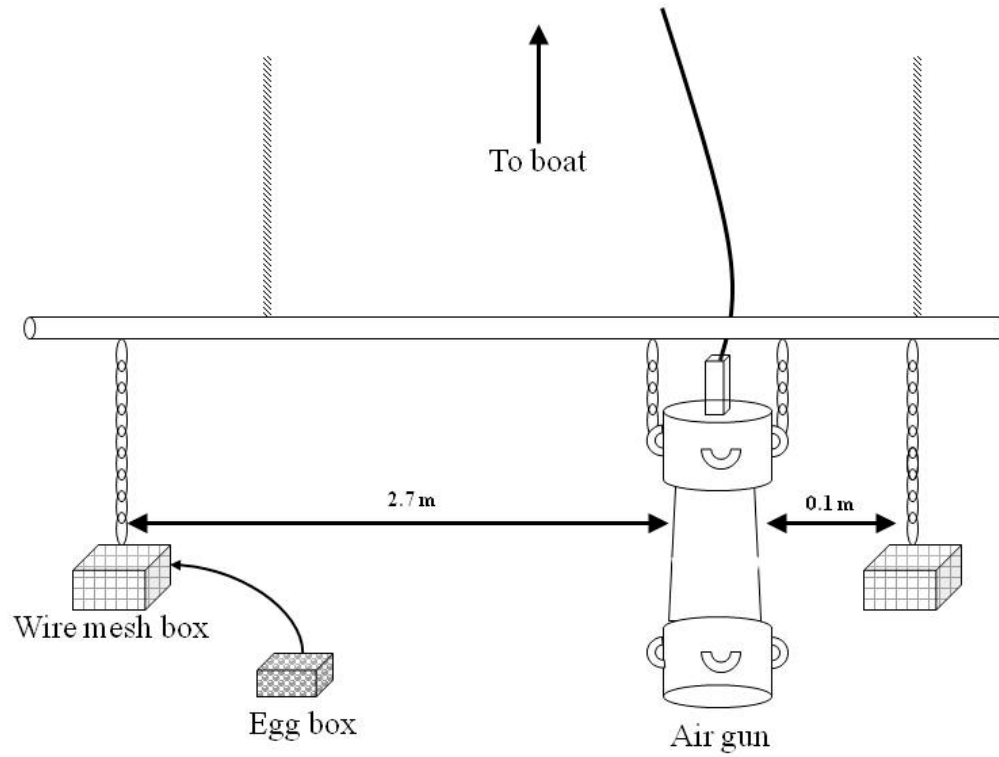


Figure 1. Diagram of apparatus used to expose lake trout embryos to a 655 cm^3 (40 in^3) air gun in Priest Lake, Idaho, autumn 2010.

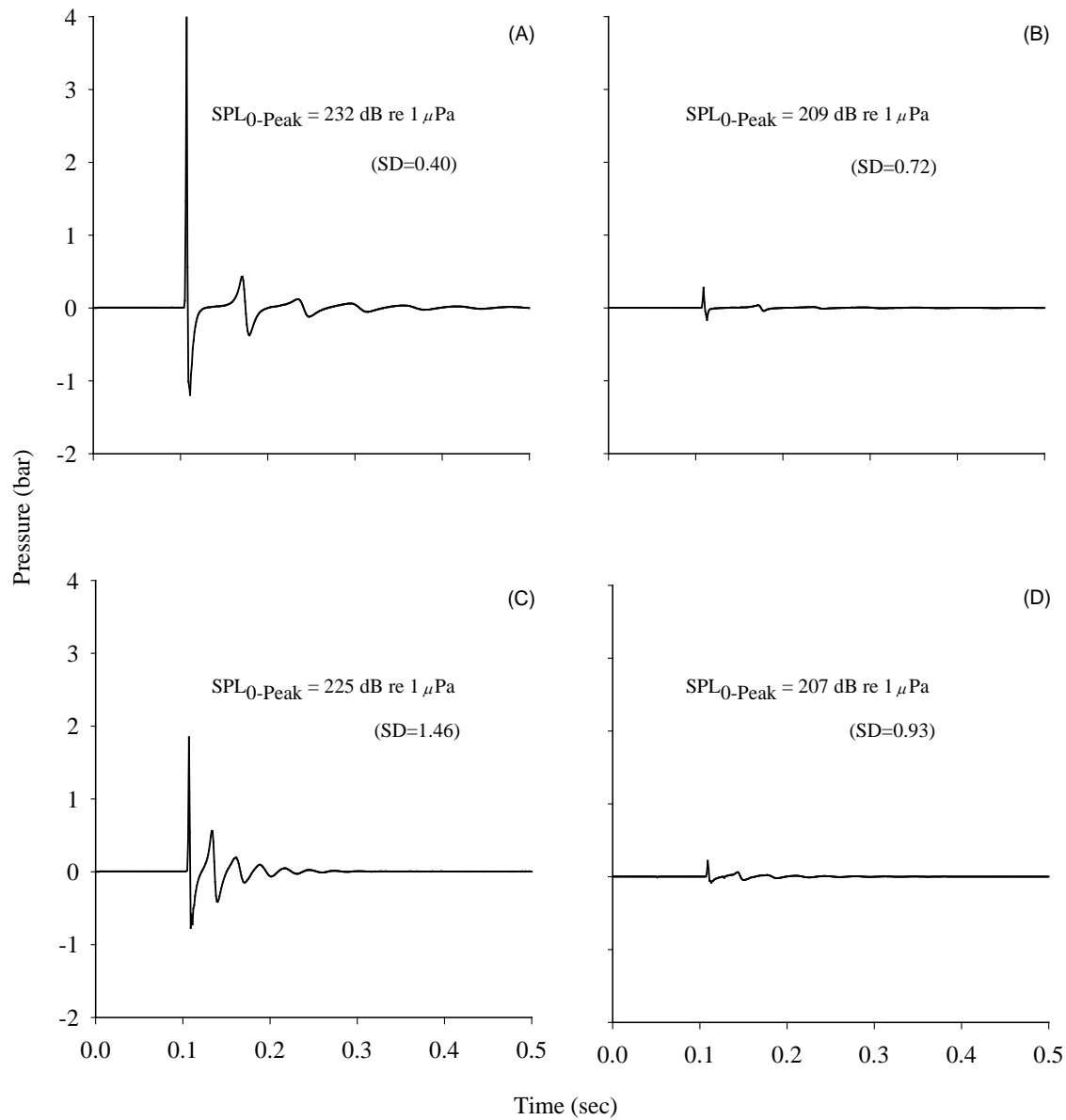


Figure 2. Pressure signatures (bar) at each treatment level of the experimental design recorded in Priest Lake, Idaho, autumn 2010. Panel A is the signature at 5 m deep, 0.1 m distance; panel B is the signature at 5 m deep, 2.7 m distance; panel C is the signature at 15 m depth, 0.1 m distance; and panel D is the signature at 15 m depth, 2.7 m distance. Calculated peak sound pressure levels and SD are given for corresponding signatures.

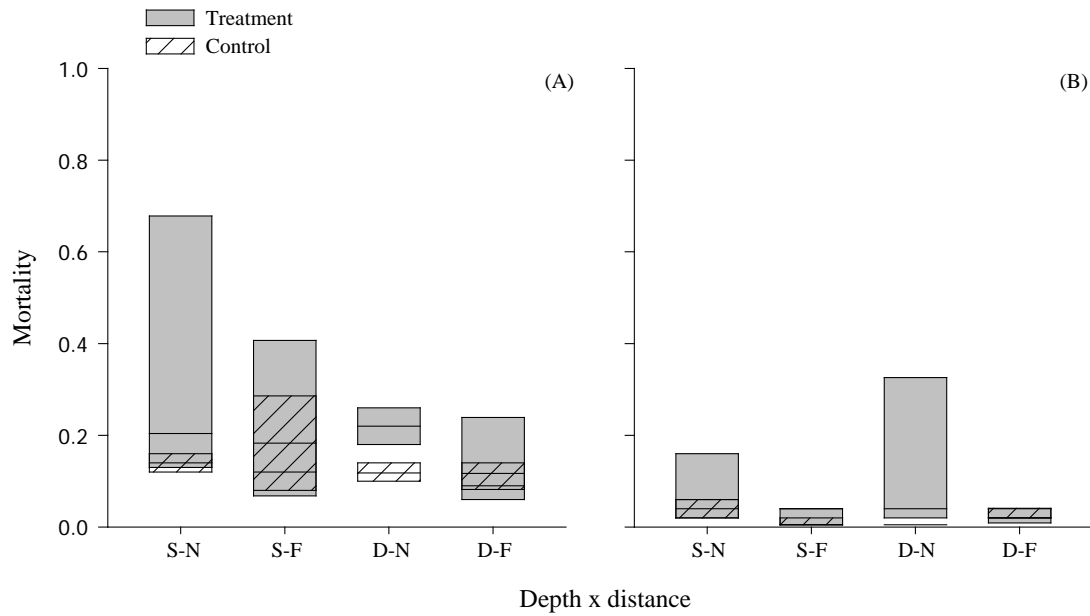


Figure 3. Box plots comparing mortality 72 h following air gun experiments on hatching rainbow trout embryos (A) and eyed kokanee embryos (B) conducted in Priest Lake, Idaho, autumn 2010. Labels on the x axis represent depth x distance treatments (S = shallow, 5 m depth; D = deep, 15 m depth; N = near, 0.1 m distance; F = far, 2.7 m treatment). Solid lines in the middle of boxes represent the median of three replicates at each treatment level. Upper and lower box boundaries represent the range of the data.

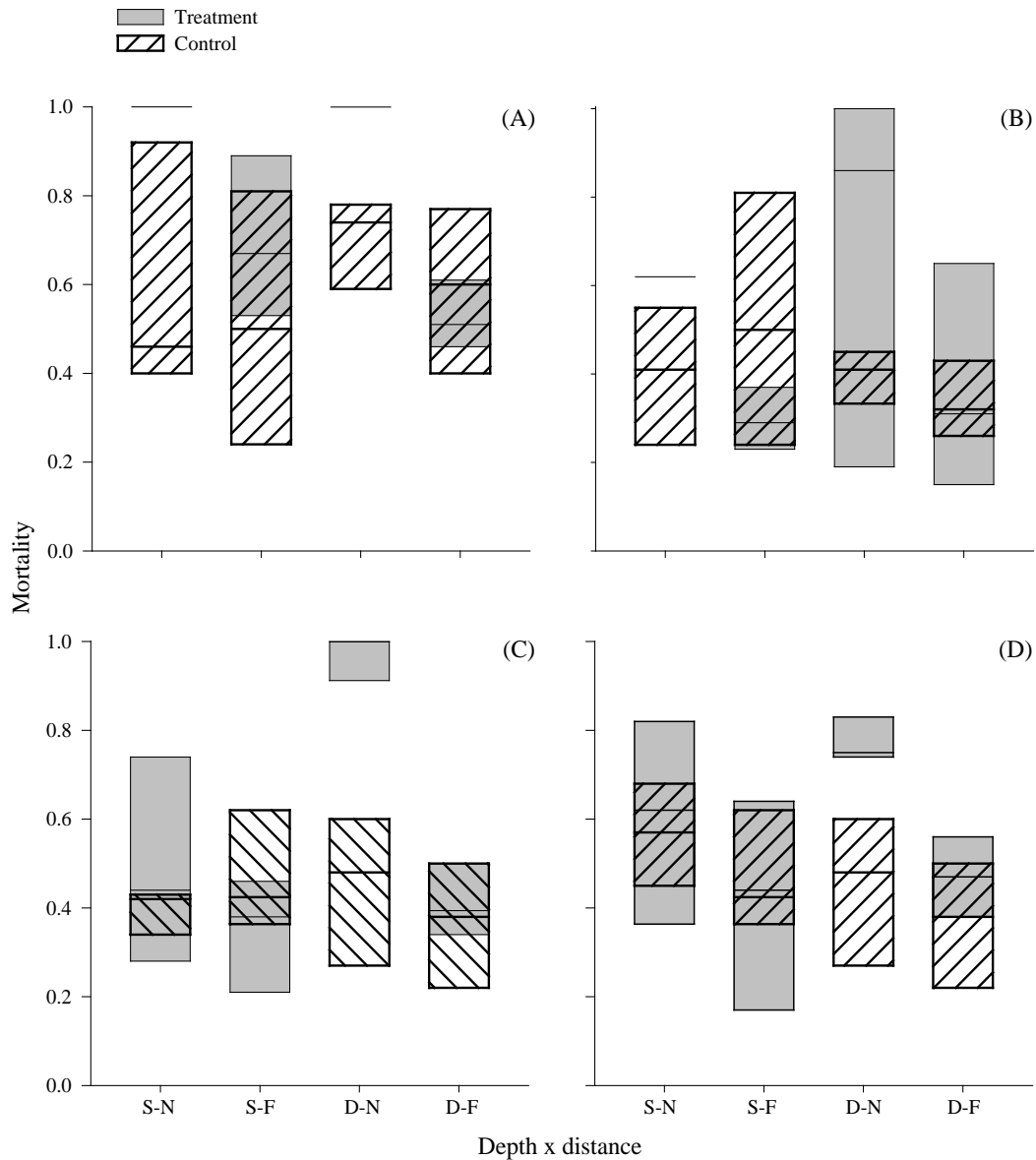


Figure 4. Box plots comparing mortality to eye-up for lake trout embryos in air gun experiments conducted in Priest Lake, Idaho, autumn 2010. Labels on the x axis represent depth×distance treatments (S = shallow, 5 m depth; D = deep, 15 m depth; N = near, 0.1 m distance; F = far, 2.7 m treatment). Panels A, B, C, and D represent experiments with embryos at 74, 156, 207, 267 TU°C, respectively. Solid lines in the middle of boxes represent the median of three replicates at each treatment level. Upper and lower box boundaries represent the range of the data.

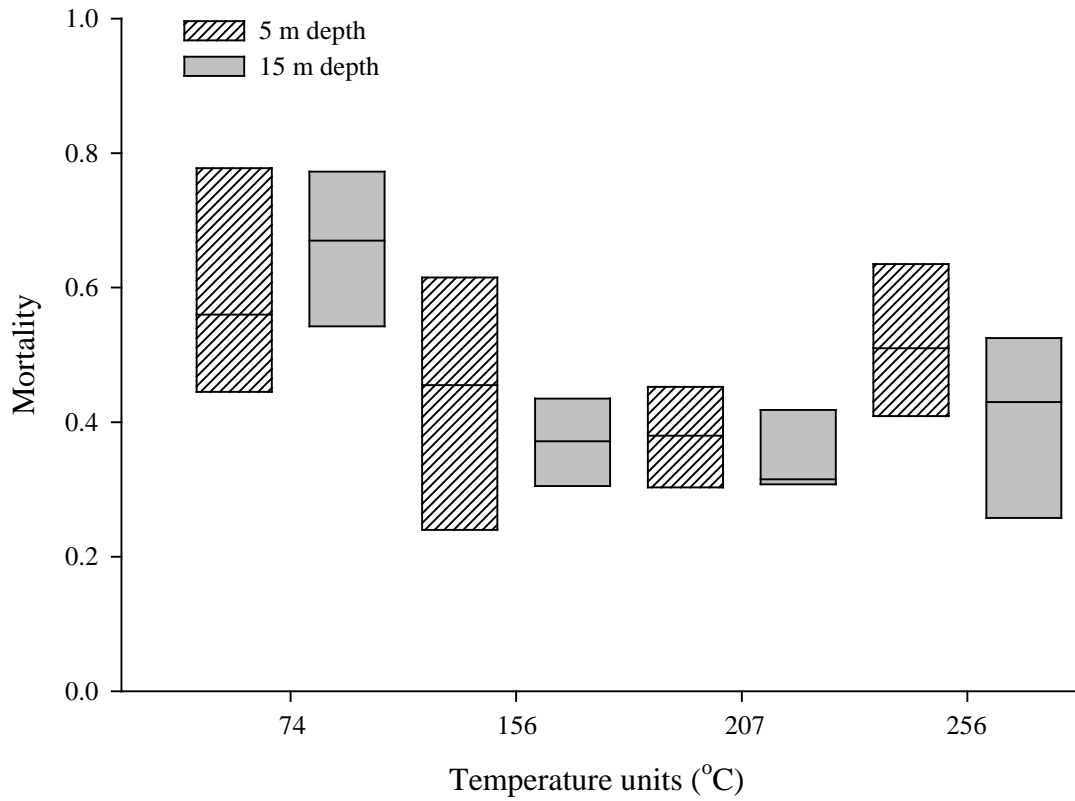


Figure 5. Box plots of mortality in controls of lake trout embryos for each depth and developmental stage from air gun experiments in Priest Lake, Idaho, autumn 2010.

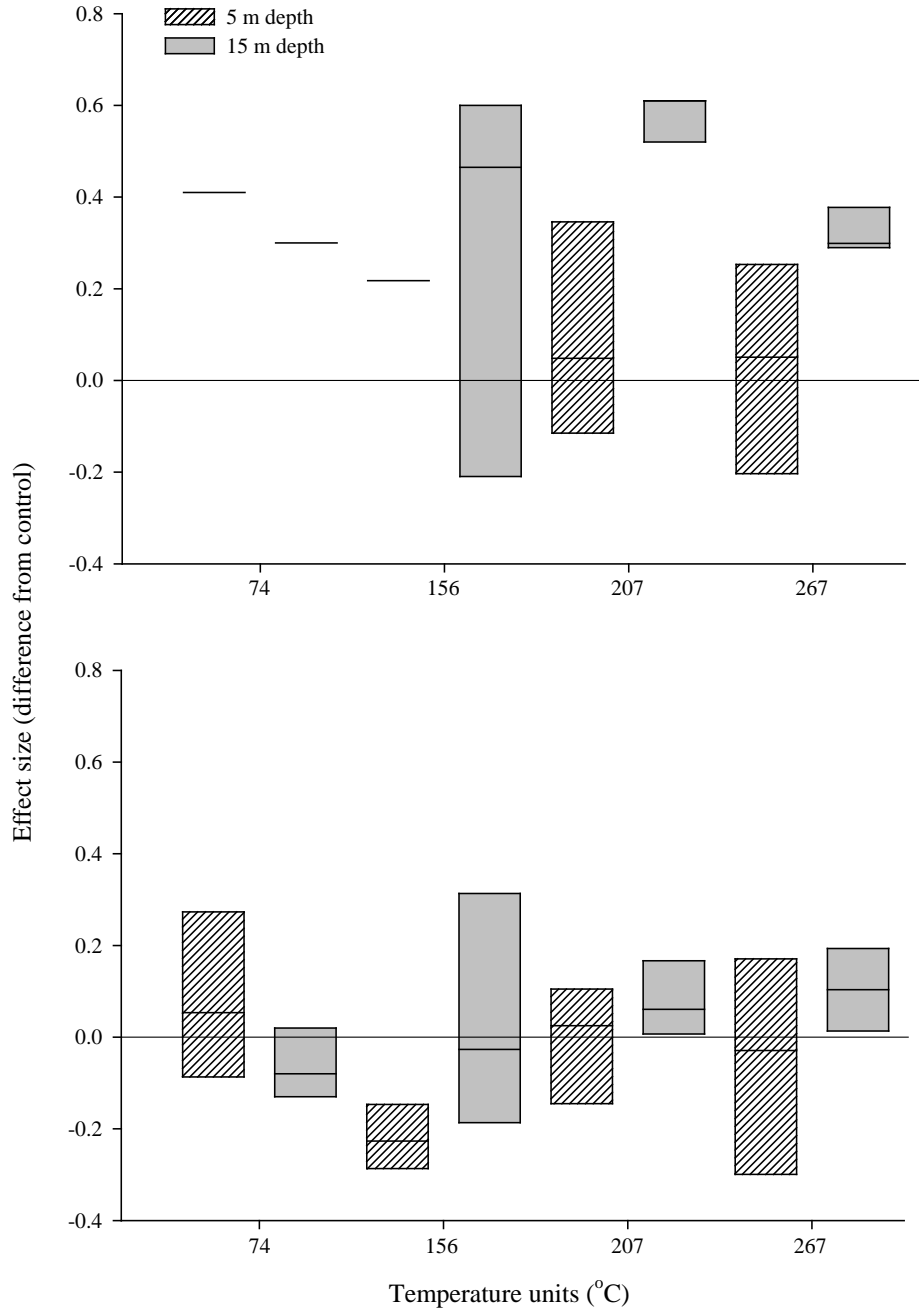


Figure 6. Box plots comparing effect sizes of 0.1 m treatments (upper panel) and 2.7 m treatments (lower panel) at two depths across developmental stages for lake trout embryos exposed to a seismic air gun in Priest Lake, Idaho, autumn 2010. Effect size was calculated as the difference of each treatment replicate from average mortality of controls in the corresponding depth and developmental stage.

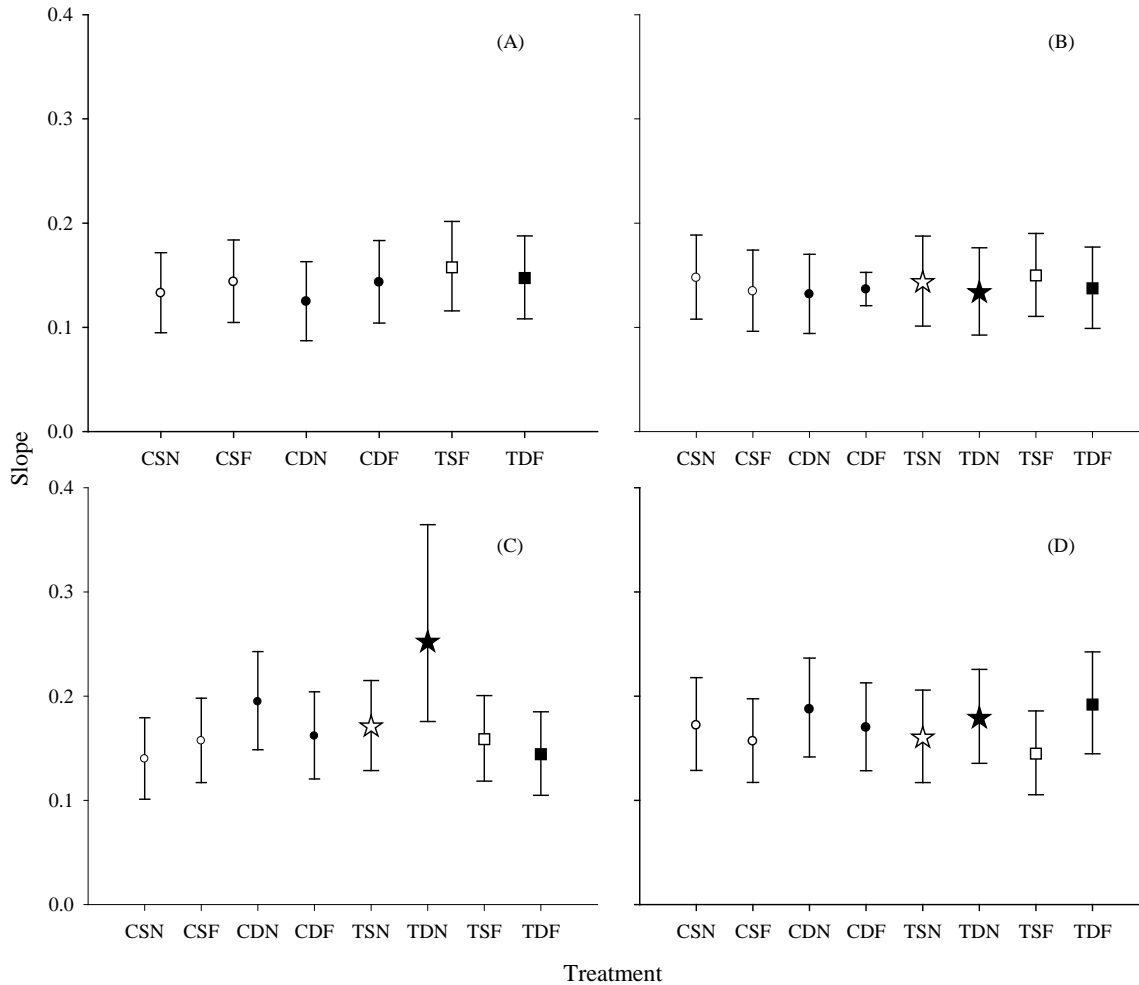


Figure 7. Estimated slopes of logistic regressions by developmental stage for lake trout embryos incubated in the University of Idaho wet lab in November 2010. Panels A, B, C, and D correspond to 74, 156, 207, 267 TU°C developmental stages, respectively. Group labels on x axis code treatment combinations, i.e., C = control, T = treatment, S (shallow) = 5 m depth, D (deep) = 15 m depth, N (near) = 0.1 m distance, F (far) = 2.7 m distance. Circles represent controls, stars represent 0.1 m distance treatments, and squares represent 2.7 m distance treatments. Open symbols are for 5 m depth treatments; filled symbols are for 15 m depth treatments.

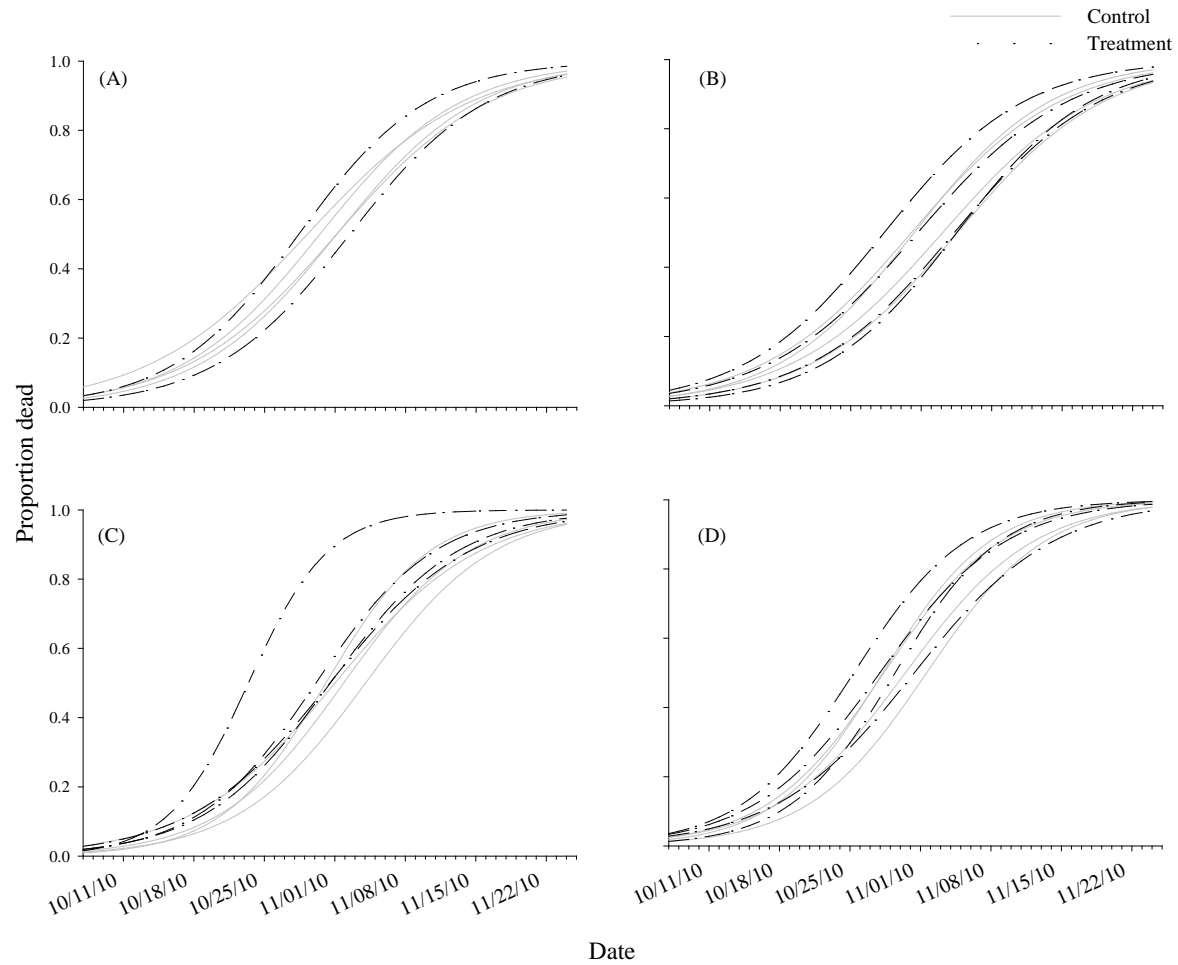


Figure 8. Logistic models of mortality through time for treatment and control groups of lake trout embryos incubated in the University of Idaho wet lab following experiments in Priest Lake, Idaho, autumn 2010. Panels A, B, C, and D correspond to 74, 156, 207, 267 TU°C developmental stages, respectively.

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