

EVALUATION OF EMBRYO SUPPRESSION METHODS FOR NONNATIVE
LAKE TROUT IN YELLOWSTONE LAKE, YELLOWSTONE NATIONAL PARK,
WYOMING, USA.

by

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ABSTRACT

Introduced Lake Trout *Salvelinus namaycush* threaten native Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* in Yellowstone Lake, Yellowstone National Park. Gill nets have been used to suppress subadult and adult Lake Trout since 1995. Because survival of embryonic and larval life history stages can have profound effects on population dynamics of Lake Trout, suppression at those stages, especially if used in concert with intensive gill netting of older fish, could enhance suppression efforts. Therefore, I conducted controlled laboratory and field experiments to systematically evaluate the effects of a variety of candidate chemical (sodium chloride, calcium carbonate, gelatin, and liquid and powdered rotenone), biological (carcass and carcass analog), and physical (sediment) suppression methods on different developmental stages of Lake Trout embryos and larvae. Liquid and powdered rotenone applications, fish carcass and carcass analog exposures, and sediment deposition significantly increased embryo mortality in laboratory experiments. Sodium chloride, calcium carbonate, and gelatin applications were not effective. *In-situ* exposure to ground carcass material in Yellowstone Lake resulted in 100% embryo mortality in 14 and 28 kg/m² biomass treatments; sediment deposition caused 97% embryo mortality among overwintering incubators. Embryo mortality was probably caused by hypoxic conditions within substrates. Embryo suppression methods differed in their effectiveness, rate at which mortality was achieved, and ease of application. These differences, as well as Lake Trout spawning site characteristics such as depth, contour, fetch, substrate size, interstitial depth, isolation, and presence of non-target organisms ultimately determine which embryo suppression method will be most applicable in a given situation. Nevertheless, implementation of successful embryo suppression techniques evaluated in this study could be used to increase mortality of Lake Trout in Yellowstone Lake. Incorporating effective embryo suppression in an Integrated Pest Management approach has the potential to provide more effective Lake Trout suppression in the long term.

EVALUATION OF EMBRYO SUPPRESSION METHODS FOR NONNATIVE LAKE
TROUT IN YELLOWSTONE LAKE, YELLOWSTONE NATIONAL PARK,
WYOMING, USA.

Introduction

Freshwater aquatic organisms are disproportionately imperiled compared to terrestrial faunal groups (Dextrase and Mandrak 2006); extinction rates of freshwater species are five times higher than those of terrestrial species (Ricciardi and Rasmussen 1999). Nonnative species are the second most common causal factor (after habitat alteration) in the extinction of freshwater fish in North America (Miller et al. 1989; Wilcove et al. 1998). Establishment of exotic species has increased throughout the world as a result of intentional, unintentional, and illegal introductions (Rahel 2000). Intentional stocking of nonnative fish was a common fisheries management practice of state and federal agencies in the United States (Rahel 2004) but has declined recently as fisheries biologists have become increasingly aware of the problems associated with nonnative fish introductions (Rahel 2000). However, illegal and inadvertent introductions continue to be a problem globally (Johnson et al. 2009).

Unauthorized illegal introductions burden agencies and society by threatening native species with extinction, damaging sustainable recreational fisheries, and diverting agency resources into expensive and often perpetual remediation programs (Pimentel et al. 2005; Johnson et al. 2009). Of particular concern are nonnative predatory fishes that are desired by the angling public (Martinez et al. 2009) but that can exert top-down

effects that can alter entire food webs, even in large lakes (McMahon and Bennett 1996). For example, Walleye *Sander vitreus*, Northern Pike *Esox lucius*, and Lake Trout *Salvelinus namaycush* recently illegally introduced in lakes of the Pacific Northwest prey upon and compete with native and sport fishes (McMahon and Bennett 1996; Koel et al. 2005; Cox et al. 2013).

Lake Trout have become widespread in the western United States because of intentional and illegal stockings and subsequent invasion events (Martinez et al. 2009). Lake Trout are large, long-lived, late maturing piscivores native to deep, cold oligotrophic lakes in Canada and the northern United States (Behnke 2002; Fredenberg et al. 2017). Lake Trout typically inhabit a top-level predator niche in lentic ecosystems (Guy et al. 2011) and often prey on valued sport and rare native fishes where they are introduced (Ruzycki et al. 2001; Koel et al. 2005). Introductions of Lake Trout can trigger trophic changes that cascade through aquatic and terrestrial food webs (Spencer et al. 1991; Ellis et al. 2011; Koel et al., in press). Through a combination of predation and disruption of ecosystem structure, Lake Trout have been implicated in native species declines and extirpations (Ruzycki et al. 2003; Vander Zanden et al. 2003). Several management agencies in the western United States have implemented suppression efforts where Lake Trout have become ecological or economic liabilities in the management of other fishes (Hansen et al. 2008; Cox et al. 2013; Hansen et al. 2016; Ng et al. 2016; Fredenberg et al. 2017). The illegal introduction of Lake Trout in Yellowstone Lake, Yellowstone National Park, Wyoming is the most highly publicized example of this fisheries resource issue (Martinez et al. 2009).

The discovery of Lake Trout in Yellowstone Lake by anglers in July 1994 raised immediate concern for the native Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* population (Kaeding et al. 1996; Ruzycki et al. 2003). The adfluvial Yellowstone Cutthroat Trout population of Yellowstone Lake is the largest remaining genetically pure inland cutthroat trout population in the world (Gresswell and Varley 1988) and is important for maintaining the integrity of the Yellowstone Lake ecosystem. Yellowstone Cutthroat Trout are a desirable food source for predators such as grizzly bears *Ursus arctos horribilis* and osprey *Pandion haliaetus* and thus serve as an important link between aquatic and terrestrial environments (Koel et al., in press). In addition to their ecological importance, Yellowstone Cutthroat Trout have considerable socioeconomic and historical significance in Yellowstone National Park (Varley and Schullery 1995). Yellowstone Lake and its outlet downstream to the Upper Falls of the Yellowstone are the most popular fisheries in the park (Varley and Schullery 1995). Nonconsumptive uses of Yellowstone Cutthroat Trout, such as viewing platforms at Fishing Bridge and LeHardy Rapids, were estimated to be used by over a third of a million visitors annually in the early 1990s (Gresswell and Liss 1995).

Because of the high ecological and socioeconomic value of Yellowstone Cutthroat Trout as well as the mandate of the National Park Service (NPS) to maintain natural ecological integrity, Lake Trout suppression is considered necessary in Yellowstone Lake (Houston 1971; McIntyre 1995). Left uncontrolled, the Lake Trout population in Yellowstone Lake was projected to cause a 90% decline in Yellowstone Cutthroat Trout numbers in 20 to 100 years (Kaeding et al. 1996). Yellowstone National

Park initiated a gillnetting suppression program in 1995 to minimize the effect of Lake Trout on Yellowstone Cutthroat Trout. Nevertheless, the Yellowstone Cutthroat Trout population of Yellowstone Lake declined precipitously (Syslo et al. 2016). The NPS contracted with a commercial fishing company, Hickey Brothers, LLC (Baileys Harbor, Wisconsin), beginning in 2009 to aid in the gillnetting effort on Yellowstone Lake. As of November 2018, >3.0 million Lake Trout had been removed from Yellowstone Lake by the gillnetting program. Despite these efforts, Yellowstone Cutthroat Trout have yet to reach primary desired management conditions as outlined for Yellowstone National Park in the Native Fish Conservation Plan (Koel et al. 2010). However, some indications of recovery have been detected. Catch-per-unit-effort of Yellowstone Cutthroat Trout in annual distribution netting increased from an average of 11.8 in 2010 to about 25 in 2018 (Koel et al. 2015; Jeff Arnold, NPS, personal communication). Additionally, Yellowstone Cutthroat Trout bycatch in Lake Trout suppression netting has increased four-fold over previous years with about 32,095 Yellowstone Cutthroat Trout caught in gill nets in 2018 (Pat Bigelow, NPS, personal communication). Bycatch in suppression netting will probably become increasingly problematic as Yellowstone Cutthroat Trout recovery continues. Accordingly, managers are seeking alternative forms of suppression that avoid bycatch.

Integrated pest management (IPM; Ehler 2006) involves synergistic use of a combination of population control methods and may be warranted for Lake Trout suppression on Yellowstone Lake (Wydoski and Wiley 1999). Successful control of undesirable fish using nets and traps alone has been limited because an insufficient

portion of the population is typically removed (Wydoski and Wiley 1999). Passive capture techniques, such as gill netting, are size selective and generally capture large fish (Hubert et al. 2012). Incorporation of novel suppression methods that actively target embryonic and larval life history stages of Lake Trout in Yellowstone Lake, in addition to gill netting large fish, may facilitate population decline. New methods, to be used in concert with the traditional techniques, are needed to achieve increased mortality of the target species (Simberloff et al. 2005; Britton et al. 2010).

Development of new control techniques requires consideration of the life history characteristics of the target species (Simberloff 2014). Baseline demographic data can inform an understanding of the population biology of the target species, thereby identifying life history stages most likely to influence population dynamics, as well as allow quantitative evaluation of the efficacy of suppression (Carslake et al. 2009; Cox et al. 2013). Demographic studies have demonstrated that Lake Trout population growth rates are most sensitive to age-0 survival rates (Ferrerri et al. 1995; Cox et al. 2013; Ng et al. 2016; Fredenberg et al. 2017). Moreover, Lake Trout are broadcast spawners that typically deposit embryos over clean porous cobble substrate with deep interstitial spaces (Gunn 1995; Marsden et al. 1995; Callaghan et al. 2015); such substrate is often spatially limited (Bigelow 2009; Cox 2010; Fredenberg et al. 2017). The scope and cost of embryo suppression may be less than those of traditional suppression programs that target later free-swimming life history stages (Brown et al. 2017).

Past research on embryonic suppression targeting Lake Trout embryos has focused on physical processes such as electricity and sound, which can kill embryonic

and larval fish (Dwyer et al. 1993; Dwyer and Erdhal 1995; Cox et al. 2012). Suppression of Lake Trout embryos with a mobile electrofishing array was evaluated on Swan Lake, Montana and Yellowstone Lake (Brown et al. 2017; Thomas 2017). The array induced mortality rates greater than 95% among embryos buried up to 20 cm in the substrate in Swan Lake (Brown et al. 2017). The array was most effective in Yellowstone Lake at the substrate surface, inducing mortality rates greater than 83% (Thomas 2017). However, the array had little effect on embryo mortality at depths of 20 cm and below because of the low specific conductance of Yellowstone Lake (Thomas 2017). High-intensity seismic air guns (developed in the 1960s to replace dynamite as a sound source in geophysical exploration; Giles 2009) discharged at 232-225 dB re 1 μ Pa at a distance of 0.1 m killed 100% of 5-d post-fertilization Lake Trout embryos at Priest Lake, Idaho (Cox et al. 2012), but discharges at a distance of 2.7 m and on later developmental stages failed to induce mortality. Unfortunately, results were confounded by high mortality among controls, which was attributed to high water temperatures (Cox et al. 2012).

Suction dredging was also evaluated as a Lake Trout embryo suppression tool for use on Yellowstone Lake (Thomas 2017). A placer gold mining dredge was used to collect cured Lake Trout eggs from experimental sites containing known densities of cured eggs. However, the proportions of eggs collected by suction dredge were low. Therefore, suction dredging was not recommended for use in embryo suppression.

Electricity, sound, and suction dredging, as used in previous studies, were limited in their applicability to reducing survival of Lake Trout embryos. Lake Trout embryos are sensitive to physical shock only during early ontogeny, with peak sensitivity

corresponding to the final stages of epiboly (Fitzsimons 1994); exposure after epiboly produces little to no mortality (Fitzsimons 1994; Cox et al. 2012). This window of sensitivity can be brief, typically ending between 150 and 176 degree days (Allen et al. 2005). Moreover, Lake Trout embryos can settle into interstitial spaces in the substrate up to 1-m deep or more (Marsden et al. 1995) beyond the effective ranges of these methods. Additionally, the electrofishing array, seismic air gun, and suction dredge are large, heavy, and cumbersome, thereby limiting the amount of spawning habitat that can be treated effectively (Cox et al. 2012; Brown et al. 2017).

The degradation of interstitial water quality of Lake Trout spawning sites has not been investigated extensively as an approach to embryo suppression. Salmonid embryos are passive recipients of prevailing water conditions (Finn 2007) and deteriorated water quality has been implicated in recruitment failure and subsequent extirpation of Lake Trout from lakes within their native range (Gunn and Keller 1984; Haines and Baker 1986). Natural degradation of spawning sites is thought to be influenced by two major processes: smothering and infilling by sediment and the decomposition of organic matter within the substrate (Sly 1988). Therefore, intentional degradation of interstitial water quality at spawning sites through the application of sediment, organic matter, or chemical compounds may efficiently and realistically reduce embryo survival.

Candidate Substances and Compounds

Chemical Compounds. Chemical compounds have been used to capture and kill fish for hundreds of years and remain the most popular method to eradicate or control undesirable fishes (Dawson and Kolar 2003; Kolar et al. 2010). Chemical means of

controlling fish populations are attractive because of ease of application, short time required to achieve lasting results, and low cost (Wydoski and Wiley 1999). However, few studies have examined the efficacy of chemical compounds in controlling embryonic life history stages of fishes, which may differ from the efficacy on older individuals because uptake of waterborne chemicals by fish is primarily achieved across the respiratory membranes of the gills (Dawson and Kolar 2003).

Rotenone. Four fish toxicants are currently registered by the U.S. Environmental Protection Agency (EPA) for use as piscicides in the United States: antimycin, rotenone, 3-trifluoromethyl-4-nitrophenol (TFM), and Bayluscide® (Olson and Marking 1975; Dawson and Kolar 2003). TFM and Bayluscide® are lampricides used to control parasitic Sea Lamprey *Petromyzon marinus* primarily in the Great Lakes region (Dawson and Kolar 2003) and therefore were not considered for Lake Trout embryo suppression. Antimycin is registered as a general piscicide and used as a nonselective fish toxicant but is currently unavailable and not used in native fish restoration actions in Yellowstone National Park. Rotenone is of primary interest in Lake Trout embryo suppression activities because it is currently widely used in both liquid and powdered formulations in native fish conservation actions in Yellowstone National Park (Koel et al. 2015) and elsewhere.

Rotenone is commonly used to control or eradicate nonnative fish in streams and lakes (Dawson and Kolar 2003). It is a natural compound isolated from the roots of certain legumes native to subtropical and tropical South America or Southeast Asia (Ott 2006). Rotenone interrupts mitochondrial electron transport, which hinders the uptake of

oxygen and leads to cell death and eventually the death of the organism (Ott 2006). Pure rotenone is insoluble in water and requires emulsifiers to effectively disperse in a waterbody. Piscicidal formulations of rotenone are typically 5% active ingredient and the remainder is composed of emulsifiers and other petroleum distillates (Ott 2006). When properly applied in a piscicidal formulation, waterborne rotenone is absorbed through the respiratory membranes of the gills (Dawson and Kolar 2003).

The toxicity of rotenone to embryonic life history stages is largely unknown. Freshly fertilized embryos of various salmonids, including Lake Trout, were more resistant than larval forms to prolonged exposures to rotenone (Olson and Marking 1975; Marking and Bills 1976). However, these were chronic exposures used to determine median lethal doses of rotenone and the specific concentrations were not reported (>1.00 mg/L). Acute rotenone exposures are more likely to simulate a realistic field application but information on acute toxicity is currently lacking. Determination of the concentrations of both liquid and powdered rotenone formulations lethal to developing Lake Trout embryos is needed to assess their potential value as alternative suppression tools on Yellowstone Lake. Additionally, the toxicity of solvent components in piscicidal formulations of rotenone to embryonic life history stages is unknown and warrants investigation.

Despite the widespread use of rotenone, investigation of more benign chemical compounds for use in Lake Trout embryo suppression is warranted from a health and safety standpoint as well as public acceptance. The majority of rotenone treatments have occurred without incident. However, a small number of projects have resulted in public

controversy and rotenone use has been limited or temporarily prohibited in some states (McClay 2000). Agencies have identified public acceptance and understanding of rotenone use as the major issues concerning its continued use. Therefore, more benign compounds could provide a less controversial alternative if proven effective at reducing embryo survival. Desirable properties of benign compounds would be those that exhibit limited potential to bioaccumulate and magnify in food chains, not be persistent, and degrade rapidly (Dawson and Kolar 2003). Additionally, candidate chemical compounds should be affordable so that largescale application on Lake Trout spawning areas is economically feasible. Three compounds were selected for further investigation as embryo suppression tools based on these criteria: sodium chloride, calcium carbonate, and powdered gelatin.

Sodium Chloride. Elevated levels of salinity in rearing waters have been shown to have adverse effects on developing salmonid embryos (Stekoll et al. 2009). High salinities are known to adversely affect development (Morgan et al. 1992; Mahrosh et al. 2014), induce ploidy alterations (Miller et al. 1994), and lower resistance to thermal stress (Craigie 1963) in both salmonid embryos and alevins. Lake Trout have a low tolerance for salinity, and are limited to waters less than 10 to 12 mg/L of salts (Behnke 2002). Salt (NaCl) is an attractive potential suppression tool because of its low cost, human safety, and ease of application. Determination of the concentrations of salt lethal to developing Lake Trout embryos is needed to determine its potential value as an alternative suppression tool on Yellowstone Lake.

Calcium Carbonate. Calcium carbonate is a naturally occurring mineral that has been implicated in limiting Lake Trout reproduction in Bear Lake, Utah and Idaho (Ruzycki 2004; Martinez et al. 2009). The lack of natural reproduction by Lake Trout in Bear Lake is not well understood, but has been attributed to limited spawning substrate, an abundance of potential egg predators, and calcium carbonate precipitates (Ruzycki 2004), which may suffocate Lake Trout embryos (Martinez et al. 2009). Calcium carbonate is inexpensive, safe to handle, and easy to apply and therefore warrants investigation as an embryo suppression tool.

Gelatin. Gelatin, the hydrolyzed form of the protein collagen, may suffocate Lake Trout embryos by inhibiting gas exchange when deposited on spawning substrates. Collagen is naturally found in the bone, skin, and connective tissue of animals. Gelatin is readily available in a powdered form, safe to handle, inexpensive, and would be easy to apply in a field setting but its efficacy as an embryo suppression technique remains untested.

Sedimentation. Facilitating natural degradation of Lake Trout spawning sites in Yellowstone Lake may be an attractive alternative embryo suppression method because the addition of a foreign chemical substance to Yellowstone Lake, no matter how benign, may be controversial. The natural degradation of Lake Trout spawning sites in the native range of the species results from deposition of fine sediment and decomposition of organic matter trapped within the substrate (Sly 1988). Fine sediment smothers embryos and the breakdown of organic matter decreases water quality in spawning substrates by

increasing biological oxygen demand (BOD), fungal and bacterial growth, and concentrations of carbon dioxide, ammonia, and hydrogen sulfide (Garside 1959; Sly 1988; Greig et al 2005). Duplication of such conditions could therefore be an embryo suppression tool.

Excessive amounts of fine sediment can reduce dissolved oxygen delivery and smother incubating embryos within spawning substrates (Argent and Flebbe 1999). Sediment cover as thin as 5 mm can markedly reduce survival in developing salmonid embryos (Kock 2004). Lake Trout generally select spawning substrates with deep interstitial spaces kept clean of fine sediment and other debris by shoreline currents or wave action (Gunn 1995; Power 2002) because sedimentation impedes the survival of naturally deposited Lake Trout embryos (Dorr et al. 1981; Manny et al. 1995; Netto 2006). Sediments rich in sand and clay in particular appear to be effective at reducing survival to hatch in incubating embryos (LaPointe et al. 2004; Greig et al. 2005). Deposition of sediment on Lake Trout spawning sites may therefore kill Lake Trout embryos in Yellowstone Lake. The most common bottom sediments of Yellowstone Lake are black obsidian sand and silty loams (Benson 1961), which could be collected and deposited on known Lake Trout spawning sites to reduce embryo survival.

Carcass and Carcass Analogs. Decomposition of organic matter within spawning substrates involves biochemical reactions associated with the breakdown of carbon, nitrogen, and sulfur compounds (Sly 1988). Suppression netting on Yellowstone Lake yields an annual catch of over 300,000 Lake Trout (Koel et al. 2015) that are killed and deposited in deep-water areas of the lake (to retain nutrients in the system and increase

handling efficiency; Koel et al. 2008). Park managers expressed interest in using this abundant resource for embryo suppression (Todd Koel, NPS, personal communication) and experimental application of whole Lake Trout carcasses in Yellowstone Lake caused high mortality of sentinel embryos (Thomas et al., in press), presumably by exposing embryos to excess nutrients, disease, or toxic substances (Sly 1988; Compton et al. 2006). However, whole carcasses drifted and attracted bears and other wildlife and placement of whole carcasses at deep water sites presents logistical challenges. Moreover, application rates required to induce embryo mortality are unknown. Application of ground (chopped) Lake Trout carcass material was proposed (Todd Koel, NPS, personal communication) to reduce drift, enhance infiltration of spawning substrate interstices, and better quantify application rates.

Widespread application of carcass material to all known Lake Trout spawning locations in Yellowstone Lake would be limited by daily gill net suppression catches during autumn because no local carcass storage facility exists. Substitution with an analogous material would alleviate this logistical constraint. Pelletized carcass analogs were used to restore nutrients in areas where anadromous salmonids historically contributed to stream productivity through decomposition (Pearsons et al. 2007). Carcass analogs evoke ecosystem responses similar to natural carcass deposition (Wipfli et al. 2004), can be produced in large quantities, are easy to transport, and pose low risk to aquatic communities (Pearsons et al. 2007). Carcass analog pellets may provide a convenient and effective means of Lake Trout embryo suppression at Yellowstone Lake.

Management actions that degrade interstitial water quality of Lake Trout spawning areas are likely to be short-term disturbances. Lake Trout generally select spawning sites on windswept shorelines where substrates are swept clean by wave action (Flavelle et al. 2002). The physical attributes that create ideal spawning habitat would also help disperse embryo suppression agents. Exposure durations achievable in the field will probably vary among candidate substances and among spawning sites. Chemical compounds may disperse at higher rates than substances such as sediment and organic matter. Therefore, achievable exposure durations need to be considered when evaluating chemical compounds and substances as embryo suppression tools in field setting.

Embryonic Developmental Progression.

Detailed knowledge of Lake Trout embryonic developmental progression in relation to water temperature could better inform optimal timing of suppression treatments because susceptibility to suppression varies among key developmental stages (Fitzsimons 1994; Cox et al. 2012). Gastrulation and early organogenesis are considered critical stages in embryonic development because the majority of natural embryonic mortality occurs during these events (Latif et al. 1999). The establishment of protein gradients that determine early embryonic cellular-fate maps occurs during gastrulation and interference with these protein signal gradients may cause development to cease (Allen et al. 2005). Although the specific mechanism of mortality during this critical stage is not well understood, any environmental stress during this period may cause increased embryonic mortality (Latif et al. 1999). The timing of reproduction, embryonic development, hatching, and swim-up influence survival of age-0 Lake Trout (Allen et al.

2005). However, sparse data exist on the developmental progression of Lake Trout embryos because field sampling during winter incubation of this autumn-spawning species is difficult. Identification of critical stages in Lake Trout embryonic development is necessary to maximize the potential effectiveness of embryo suppression treatments.

Lack of suppression techniques that target embryonic and larval life history stages of Lake Trout probably impedes successful management of nonnative Lake Trout populations in the western United States because suppression at these stages can have profound effects on population dynamics (Ferrerri et al. 1995). Moreover, the need for novel suppression techniques will continue to grow as nonnative species spread globally (Crowl et al. 2008, Simberloff 2015) and the per-fish costs of traditional suppression methods increase (Brown et al. 2017). Development of embryonic suppression methods to be used synergistically with intensive gill netting has been recommended for Lake Trout suppression in Yellowstone Lake and elsewhere (Cox 2010; Gresswell et al. 2015; Brown et al. 2017). My objectives were therefore to systematically evaluate the effects of a variety of candidate chemical (sodium chloride, calcium carbonate, gelatin, and liquid and powdered rotenone), physical (sediment), and biological (carcass and carcass analog) suppression methods on different developmental stages of Lake Trout embryos and larvae in controlled laboratory and field experiments and thereby help inform the development of Lake Trout embryo suppression methods for use on Yellowstone Lake and other waters where Lake Trout suppression is desirable.

Study Area

Laboratory Trials

The Bozeman Fish Technology Center (BFTC) is located on Bridger Creek about 6.4 km northeast of Bozeman, Montana. Laboratory trials were performed in the hatchery building at the BFTC in vertical flow incubators and aluminum flow-through troughs. The hatchery facility is supplied with cold (7-8°C) and warm (20-22°C) spring water that allows incubation at temperatures ranging from 8-22°C.

Field Trials

Yellowstone Lake is located in northwest Wyoming in the east-central portion of Yellowstone National Park. Yellowstone Lake lies at an elevation of 2356 m near the headwaters of the Yellowstone River and is isolated by large waterfalls downstream of its outlet. The lake is the largest alpine lake in North America (341 km²; Kaplinski 1991). Yellowstone Lake has a mean depth of 48 m and a maximum depth of 107 m (Kaplinski 1991). Ice cover typically occurs from late December through May, and the lake becomes thermally stratified in July with a thermocline developing at a depth of 15 m (Koel et al. 2008). Hypolimnetic waters below the thermocline remain well oxygenated throughout the summer and surface water temperature rarely exceeds 18°C (Benson 1961). Two native fishes and four established nonnatives inhabit Yellowstone Lake. Yellowstone Cutthroat Trout and Longnose Dace *Rhinichthys cataractae* are native (Koel et al. 2005). Longnose Suckers *Catostomus catostomus*, Lake Chub *Couesius plumbeus*, Redside Shiners *Richardsonius balteatus*, and Lake Trout were introduced (Koel et al. 2005).

Fieldwork was conducted at three Lake Trout spawning sites: Carrington Island, Thomas Bank, and Olson Reef (Figure 1).

Methods

Embryo Collection

Embryos were obtained from three different sources. Lake Trout embryos were collected from hatchery broodstock at Saratoga National Fish Hatchery, Saratoga, Wyoming, and wild-caught adults from Yellowstone Lake. Lake Trout embryos are seasonally restricted in their availability to autumn, and those produced in fish hatcheries are often in demand and allocated to state stocking programs. Because of their similar structure and greater availability, Rainbow Trout *Oncorhynchus mykiss* embryos were used as surrogates for Lake Trout embryos in summer laboratory trials. Rainbow Trout embryos were obtained from Ennis National Fish Hatchery, Ennis, Montana. Rainbow Trout embryos were used primarily to screen candidate suppression techniques for subsequent evaluation with Lake Trout embryos.

Hatchery-sourced embryos were obtained on the day of spawning and transported immediately by motor vehicle in 1-L thermoses held in 150-L coolers to the BFTC. Upon arrival, embryos were slowly acclimated to hatchery water temperatures by floating the thermoses in large plastic bins filled with hatchery water. Embryos were reared in 8-tray vertical flow incubators (MariSource). Embryos were further subdivided within Heath trays into PVC wells with each well holding 100 embryos. Wells were made of 7.6 cm

diameter PVC pipe cut to length and sealed on one end with window screen affixed with waterproof contact adhesive.

Wild-caught adult Lake Trout were obtained from NPS suppression nets on Yellowstone Lake during spawning season (September-October). Live fish were removed from gill nets and held in plastic holding tanks filled with lake water until a sufficient number of male and female Lake Trout were collected (about 4 individuals of each sex) for gamete collection and fertilization (Pennell and Barton 1996). Multiple female and male Lake Trout were crossed simultaneously to avoid deleterious familial effects. Female Lake Trout were blot dried, and their eggs were stripped into a dry container by applying pressure along the abdomen in an anterior to posterior direction. Milt was collected from male Lake Trout similarly and mixed with the eggs. Lake water was added to the container to activate the milt, and a feather was used to gently mix the gametes. The container was held out of direct sunlight for about 10 minutes to allow fertilization. Fertilized eggs were rinsed in lake water using a sieve and transferred to 1-L thermoses filled with lake water. Thermoses were stored on ice within larger 150-L coolers and transported to the NPS Aquatic Sciences office at Lake Village. Embryos for laboratory trials were transferred to the BFTC as described above. Embryos for field trials were reared in Heath trays (suspended screens) within Living Stream System[®] (Frigid Units, Inc.) tanks at 9°C.

Laboratory Trials

Embryonic Developmental Progression. Daily embryonic developmental progression of Lake Trout embryos was monitored to determine the timing of critical

stages in embryonic development. Four replicates of 1,200 embryos were allocated to embryonic developmental monitoring. Replicates were split into two groups reared at 8°C and 10°C. Temperatures were selected based on reported values that would expedite developmental progression (Allen et al. 2005). Ten individual embryos were removed daily from each Heath tray and preserved in formalin. Embryonic development of preserved embryos was described after examination by light microscopy and photographed to create a photo series of daily developmental progression.

Chemical Compounds. Controlled laboratory experiments were performed to systematically evaluate the effects of a variety of candidate chemical compounds on different developmental stages of Lake Trout embryos and larvae to help inform the development of Lake Trout embryo suppression methods for use on Yellowstone Lake. Lake Trout embryos and larvae were reared at 8 and 10°C and Rainbow Trout embryos were reared at 13°C. A factorial experimental design was used to examine how chemical concentration, exposure duration, and developmental stage influence mortality (Table 1). Static chemical exposure trials were performed in 11.4-L exposure boxes. Exposure boxes were filled with 6 L of hatchery water. Chemicals to be evaluated were added to the 6 L of water and mixed evenly using a stir stick. Wells containing embryos or larvae to be exposed were removed from Heath trays, dewatered momentarily, and submerged in the exposure boxes as expediently as possible without causing unnecessary physical disturbance. Lids were secured over the exposure boxes after wells were submerged. Exposure boxes were placed into aluminum flow-through culture tanks supplied with hatchery water for the duration of the exposure to maintain stable temperatures within the

static exposure boxes. Embryos and larvae were exposed to chemical compounds for 4 or 12-hour durations. Acute exposure durations of 4 and 12 hours were selected because fine scale application of chemical compounds in a large lentic system is likely to disperse relatively rapidly. When exposure duration was elapsed, wells were briefly dewatered from exposure boxes and returned to vertical incubators where embryonic and larval mortality was monitored until the termination of the experiment. Two developmental stages of embryos were exposed to chemicals, an early group at the end of gastrulation and a later group in early organogenesis. Developmental stages selected for chemical exposure were based on critical stages observed in the development of other teleost fishes (Latif et al. 1999; Allen et al. 2005). Larvae were exposed one week after completion of hatch (600 degree-days). Embryos exhibiting slight marbling or complete opaqueness were considered dead and removed by pipette to avoid fungal outbreaks among survivors. Embryo chemical exposures were terminated upon completion of hatch and larval chemical exposures were terminated at the swim-up stage (700 degree days; Janssen et al. 2007). All remaining larvae were euthanized in an overdose of MS-222.

Liquid and Powdered Rotenone. Exposure to liquid and powdered formulations of rotenone was evaluated at two concentrations to determine effectiveness as an embryo suppression tool. Doses of 2 and 4 mg/L of both rotenone formulations were evaluated in embryonic chemical exposures. Concentrations of rotenone formulations selected for experimental use were based on legal limits established by the EPA.

Rotenone Solvents. The piscicidal formulation of rotenone that Yellowstone National Park uses in native fish restoration, CFT Legumine®, contains two main solvents for the dispersal of rotenone in water: N-methylpyrrolidone (NMP) and diethylene glycol ethyl ether (DGEE) (Ott 2006). The toxicity of these two solvents was evaluated to determine their partial contributions to observed embryo mortalities in rotenone treatments. NMP toxicity was evaluated at doses of 0.2 and 0.4 mg/L. DGEE toxicity was evaluated at doses of 1.3 and 2.6 mg/L. Solvent doses selected were based on their corresponding concentrations in 2 and 4 mg/L rotenone exposures.

Sodium Chloride. Two concentrations of sodium chloride, 2,500 and 5,000 mg/L, were evaluated on Lake Trout embryos (Table 1). Concentrations of sodium chloride selected for experimental use are based on chronic exposure levels to elevated salinities that resulted in reduced hatching success in multiple fish species (Koel and Peterka 1995).

Calcium Carbonate. Calcium carbonate exposures were performed at four concentrations. Rainbow Trout embryos were exposed to 268 and 1,000 mg/L doses of calcium carbonate. These concentrations were based on naturally occurring levels of calcium carbonate from Bear Lake, Utah and Idaho, where the substance is implicated in limiting Lake Trout recruitment (Palacios et al. 2007). Concentrations used for Lake Trout embryo exposures were increased (e.g. 10,000 and 50,000 mg/L) in attempts to elicit a treatment effect.

Gelatin. Gelatin was evaluated by exposing developing embryos to four concentrations. Rainbow Trout embryos were exposed to 5,833 and 8,166 mg/L doses of gelatin. Lake Trout embryos were exposed to 500 and 1,000 mg/L doses of gelatin. Concentrations selected for Rainbow Trout embryo exposures were based on the amount of powdered gelatin required to create a 1 and 5 mm gelatinized layer within exposure boxes. Amounts in Lake Trout exposures were reduced to determine if a lower dose could produce similar results to that of Rainbow Trout embryo exposures.

Carcass and Carcass Analogs. Various carcass and carcass analog experiments were performed to investigate different aspects of carcass application on embryo mortality. Exposures to whole fish carcasses were performed with both Rainbow Trout and Lake Trout embryos. Whole carcass application is desirable to managers because it would minimize handling time during embryo suppression efforts. Embryo exposures under static conditions to 10-g pieces of carcass musculature were performed using both Rainbow Trout and Lake Trout embryos. Static exposures were performed to determine how lack of water exchange influences embryonic mortality during carcass exposure. Exposures to ground carcass and carcass analog pellets were performed using Rainbow Trout embryos.

Rainbow Trout carcasses were used in laboratory experiments to avoid disease issues associated with the transfer of Lake Trout from Yellowstone Lake to the BFTC. Rainbow Trout maintained at the BFTC were euthanized in an overdose of MS-222 and rinsed prior to initiation of carcass experiments. Sizes of Rainbow Trout used in carcass

experiments were dependent on availability at the time experiments took place and ranged from 200 to 600 mm.

Carcass material exposures were chronic to duplicate expected conditions experienced by embryos in Yellowstone Lake (Thomas et al., in press). Exposure duration extended from blastulation to hatch. Mortality, temperature, and dissolved oxygen concentration were monitored daily. Embryos exhibiting slight marbling or complete opaqueness were considered dead and removed by pipette to avoid fungal outbreaks that could affect other embryos and confound results.

Whole Carcass. Whole carcass material exposures were vertically tiered in three separate trays in vertical flow incubators. Three control embryo wells containing 100 embryos each were placed in the top-most tray, the middle tray received one whole Rainbow Trout carcass, and the bottom tray held three treatment embryo wells containing 100 embryos each. The influence of water exchange on embryo mortality was investigated by performing whole carcass experiments at multiple flow rates. Incoming single-pass well water was set to 7.6 L/min, 3.8 L/min, or 1.9 L/min in three vertical flow incubators in Rainbow Trout embryo exposures. Flow rates were set to 0.8 L/min, 0.4 L/min, or 0.2 L/min in Lake Trout embryo exposures. Initial flow rates were selected based on incremental reduction of optimal flows for vertical flow incubators (7.6 L/min). Subsequent flows selected for Lake Trout embryo exposures were based on results from initial Rainbow Trout embryo exposures.

Static Carcass. Static carcass exposures were performed in exposure boxes within a temperature controlled environmental chamber. Exposure boxes each received one L of water. Each exposure box contained two glass petri dishes. One petri dish received 10 embryos and the other received a 10-g piece of Rainbow Trout muscular tissue (treatment) or nothing (control). Three controls and three treatments were housed in the environmental chamber. Oxygen bubblers were added to exposure boxes in Lake Trout embryo trials to determine the role dissolved oxygen plays in embryo mortality in carcass exposures. Dissolved oxygen concentrations were maintained above 9 mg/L for the duration of the experiment in controls and treatments.

Ground Carcass. Ground carcass material exposures were vertically tiered in eight separate trays in vertical flow incubators. Unlike whole carcass experiments, ground carcass experiments evaluated embryo mortality at different levels of carcass material biomass (Table 1) to determine minimum amount of carcass material required for effective embryo suppression. Embryos were exposed to four levels of ground carcass material: 5 kg/m², 10 kg/m², 14 kg/m², and 19 kg/m². Heath trays within a vertical flow incubator alternated between trays containing carcass material and trays containing ten embryo wells with 100 embryos each. This design allowed for compounding exposure to ground carcass material as water descends through the vertical flow incubator. Control embryos were housed similarly in a separate vertical flow incubator with no added ground carcass material.

Carcass Analogs. Carcass analog material was experimentally evaluated using Rainbow Trout embryos in trials structured similarly to ground carcass material experiments. Fish-protein and plant-protein based carcass analog pellets were produced and assessed as surrogates for carcass material. Exposures were vertically tiered in vertical flow incubators as for ground carcass trials. Exposures to four levels (5 kg/m², 10 kg/m², 14 kg/m², and 19 kg/m²) of carcass analogs were evaluated to determine minimum levels of biomass required for embryo suppression. Heath trays within vertical flow incubators alternated between trays containing carcass analogs and trays containing ten embryo wells with 100 embryos each so that exposure levels compounded as water descended through the incubators.

Rainbow Trout carcass analog pellets were produced to act as a surrogate material for fish carcasses and evaluated as an embryo suppression tool. Twelve kilograms of Rainbow Trout from the Bozeman Fish Technology Center were euthanized in an overdose of MS-222 and rinsed to serve as the foundation of the carcass analog pellets. Rainbow Trout carcasses were ground into a paste (meal) using a commercial food cutter (Hobart®). The addition of binding agents (soy meal, wheat flour, and wheat gluten) was required to facilitate proper pelletizing because of the high oil content of the Rainbow Trout meal. The composition of the resulting mixture was as follows: 50% soy bean meal, 45% Rainbow Trout meal, 0.25% wheat flour, and 0.25% wheat gluten meal. Prior to mixing, all ingredients were ground using an air-swept pulverizer (Jacobsen 18H, Minneapolis, Minnesota, USA) for 100% pass through a screen with 250 µm openings. Dry ingredients were mixed in a horizontal paddle mixer (Marion Mixers, Marion, Iowa)

where ~30% weight by weight (w/w) moisture was added to the mix. Pellets were formed using a water-cooled extrusion system (Italgi Model No. P35A, Carasco, Italy). Particle sizes of 3 mm diameter were produced and dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland) with air discharge temperature remaining below 102°C to obtain a final moisture content of less than 8% as determined by microwave moisture analyzer (Sartorius-Omnimark uWave, Bohemia, New York).

Plant-based carcass analog pellets were produced to determine if a carcass analog lacking fish-based protein can be used as an effective embryo suppression tool. Plant-based carcass analog pellets were made of 67% soy protein concentrate, 18% soy oil, 10% soy bean meal, 2.5% wheat flour, and 2.5% wheat gluten meal formula. This formula was based on the protein and lipid content of Rainbow Trout carcass analog pellets. The ingredients were processed in the same manner as the Rainbow Trout carcass analog pellet mixture.

Sedimentation. Laboratory sedimentation trials were performed to determine embryo mortality at different levels of sediment cover. Sediments were obtained by suction dredge from Yellowstone Lake near Carrington Island. Particle size was estimated by running samples through a series of sieves (6.3, 2.0, 1.0 and 0.5 mm). Particle size analysis was used to create a sediment profile for laboratory use. Sedimentation trials were conducted in five aluminum hatchery troughs supplied with single-pass well water at 13°C. Embryo incubation units used in these trials were similar to those used in previous embryo sedimentation experiments (Kock 2004). Incubators within each trough were randomly assigned a sedimentation level of 0, 5, 10, 15, or 20

mm. Each incubator received 10 Rainbow Trout embryos. Two durations of sediment cover (10 and 20 d) were used to assess short and long term effects of sediment deposition. Mortality was calculated for each incubator upon completion of exposure duration.

Field Trials

Ground Carcass. The efficacy of ground carcass material application for Lake Trout embryo suppression was evaluated at Thomas Bank in Flat Mountain Arm and Olson Reef (Figure 1). Fertilized embryos were transferred to plastic grid incubators at the NPS Aquatic Sciences office and placed into 150-L coolers for transport to field sites by boat. Each plastic grid incubator received 50 fertilized embryos. Divers using SCUBA gear placed plastic grid incubators within the substrate at twelve pre-delineated 3×3 m shallow (1.5 to 3 m) water sites at Thomas Bank in Flat Mountain Arm. Experimental sites were delineated using polypropylene rope with 4.5-kg weights at each corner. Twelve plastic grid incubators were placed in each 3×3 m site (Thomas et al., in press). MiniDot loggers (PME, Inc.) were placed at sites to monitor dissolved oxygen concentrations and temperatures. Lake Trout carcasses were collected from ongoing NPS Lake Trout suppression efforts and processed using a CHP-H22 Piranha Bait Chopper (Yaquina Boat Equipment, Inc.). The twelve 3×3 m sites were divided into four groups of three. Three sites served as control sites, three received 250 kg of ground carcass material, three received 125 kg, and three received 62.5 kg. Lake Trout carcass material was deposited by dumping plastic bins with pre-weighed ground carcass material directly onto study plots from the research vessel. A subset of plastic grid incubators was

collected in late October to assess short-term effects on Lake Trout embryonic mortality (n=6). All remaining grid incubators remained in place and were collected the following June to assess long-term effects.

The addition of ground Lake Trout carcass material may influence nutrient loading at spawning sites (Thomas et al., in press). Measurement of substrate biofilm was used to quantify the effect of carcass material deposition at experimental sites in Yellowstone Lake. Biofilm dry biomass was quantified before and after carcass deposition at experimental sites. Biomass was determined by scraping biofilm off of three rocks of similar size per site and measuring the surface area of each rock using the foil method (Morin 1987). A known amount of the resulting slurry was homogenized then filtered through a pre-weighed glass-fiber filter (Fetscher et al. 2009). The filtered sample was dried for 24 hours at 105°C and then weighed to obtain dry biomass. Surface area and dry biomass measurements were used to calculate biofilm biomass per unit area for each rock (Hauer and Lamberti 2011).

The efficacy of deep-water spawning site carcass treatment was evaluated at Olson Reef. Lake Trout embryos were not used in these experiments because of difficulty associated with deep-water diving at high altitude. Water chemistry parameters were monitored to determine if similar negative influence of carcass deposition can be achieved at depth. Researchers placed ground carcass material on spawning substrates at Olson Reef. Dissolved oxygen concentrations and temperature were monitored at the substrate surface using a MiniDot logger.

Sedimentation. Sedimentation field trials were conducted at Carrington Island in the West Thumb of Yellowstone Lake (Figure 1). Spawning areas surrounding Carrington Island were experimentally divided into two halves, with the north half considered the treatment area and the south half considered the control. Three 3×3 m experimental sites were established in each half. This nonrandom assignment was designed to minimize transfer of sediment from treatment to control sites by prevailing winds and fetch. Sites were delineated and incubators allotted as in ground carcass field trials. Sediment depth gauges were placed within the substrate at each treatment site to determine depth of sediment deposited and monitor site recovery rates. MiniDot loggers (PME, Inc.) were also placed at sites to monitor dissolved oxygen concentrations and temperatures. Nearby fine sediment was collected using a Piranha Mini-Dredge Model PS165E (Piranha Pumps, Inc.) suction dredge operated by NPS crews and deposited onto the treatment area of the Carrington Island spawning substrates. A subset of plastic grid incubators from each site was collected in late October to assess short-term effects of sedimentation on Lake Trout embryo mortality ($n=6$). The remaining plastic grid incubators were collected the following June to assess long-term effects.

Data Analyses

All statistical analyses and model diagnostics were performed and evaluated in R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria). Values were considered significant at $\alpha < 0.05$.

Laboratory Trials. Chemical trial data were analyzed using three-way analysis of variance (ANOVA; package ‘car’) to detect differences in mean embryonic mortality at different chemical concentrations and exposure durations for the two developmental stages. A Tukey-Kramer test (package ‘multcomp’) was used for pairwise comparisons among different levels of concentration, exposure duration, and developmental stage. Two-way ANOVA was used to analyze results from whole carcass, ground carcass, and carcass analog experiments. A Tukey-Kramer test was used for pairwise comparisons among different levels of biomass and treatment type. A two-sample t-test was used to detect differences in mean embryonic mortality between control and exposure embryos in static carcass exposures. Sedimentation trial data were analyzed using two-way ANOVA to detect differences in mean embryonic mortality at different depths of sediment cover and exposure durations. A Tukey-Kramer test was used for pairwise comparisons among different levels of sediment depth and treatment type.

Field Trials. Mortality data from ground carcass field trials were analyzed using two-way ANOVA to detect differences in mean embryonic mortality at different levels of deposited carcass biomass and embryo depth within the substrate. A Tukey-Kramer test was used for pairwise comparisons among different levels of biomass, depth, and treatment type. Biofilm data were analyzed using a paired t-test to detect differences in mean change in biofilm before and after carcass material application at both control and treatment sites. Mortality data from sedimentation field trials were analyzed using two-way ANOVA to detect differences in mean embryonic mortality at different levels of

sediment cover and embryo depth within the substrate. A Tukey-Kramer test was used for pairwise comparisons among different levels of depth and treatment type.

Results

Laboratory Trials

Embryonic Developmental Progression. Daily embryonic developmental progression of Lake Trout embryos agreed with the timing of stages outlined by Allen et al. (2005) with the following timing of key developmental stages: blastulation was complete by 66 degree days, epiboly occurred between 66 and 146 degree days, gastrulation between 134 and 228 degree days, and organogenesis between 207 and 518 degree days. Daily mortality of Lake Trout embryos increased during two developmental stages: gastrulation and organogenesis (Figure 2). Embryo mortality was initially low but increased at 11 days post fertilization (DPF) among embryos reared at 10°C and at 13 DPF among embryos reared at 8°C. Mortality peaked at 6.1% at 13 DPF (130 degree days) among embryos reared at 10°C and at 5.1% at 16 DPF (128 degree days) among embryos reared at 8°C. Peak mortality occurred with the onset of gastrulation. Daily mortality decreased to below 1% following peak mortality but increased again later in development. Mortality increased during organogenesis to 2.0% at 36 DPF among embryos reared at 10°C (360 degree days) and to 2.3% at 46 DPF among embryos reared at 8°C (368 degree days).

Chemical Compounds

Liquid Rotenone. Exposure to liquid rotenone for 12 h increased mortality of Lake Trout embryos, but exposure for 4 h did not (Figure 3). Almost all embryos exposed to 4 mg/L liquid rotenone for 12 h died, including embryos exposed at both 8 DPF (mean, 98%; 95% CL, 95.5 and 100%) and 33 DPF (mean, 96%; 95% CL, 92.3 and 99.9%). Mean percent mortality differed between embryos exposed to 2 mg/L liquid rotenone for 12 h at 8 DPF (mean, 59%; 95% CL, 49.9 and 69.1%) and 33 DPF (mean, 81%; 95% CL, 73.9 and 89.1%); pairwise comparisons revealed both means were detectably higher than those of corresponding controls ($P < 0.05$). A three-way interaction among treatment, exposure duration, and developmental stage (ANOVA: $F_{2, 59} = 14.27$, $P < 0.001$) indicated that as liquid rotenone concentration increased the influence of exposure duration on embryo mortality was greater at the late developmental stage.

Exposure to liquid rotenone for 12 h increased mortality of Rainbow Trout embryos, but exposure for 4 h did not (Figure 4). Embryo mortality was highest following 4 mg/L liquid rotenone exposures for 12 h at both 5 DPF (mean, 62%; 95% CL, 52.5 and 71.5%) and 16 DPF (mean, 49%; 95% CL, 39.5 and 59.1%). Mean percent mortality of embryos exposed to 2 mg/L liquid rotenone for 12 h was greater at 5 DPF (mean, 40%; 95% CL, 30.7 and 49.9%) than at 16 DPF (mean, 22%; 95% CL, 14.2 and 30.5%). No evidence supported a three-way interaction among treatment, exposure duration, and developmental stage (ANOVA: $F_{2, 24} = 0.73$, $P = 0.49$). A two-way interaction between treatment and exposure duration indicated that as liquid rotenone

concentration increased embryo mortality was greater following 12-h exposure durations than after 4-h exposure durations (ANOVA: $F_{2, 24} = 25.82$, $P < 0.001$).

Powdered Rotenone. Exposure to powdered rotenone for 12 h increased Lake Trout embryonic mortality, but 4-h exposures did not (Figure 5). Almost all embryos exposed to 4 mg/L powdered rotenone for 12 h at 26 DPF died (mean, 99%; 95% CL, 97 and 100%); mortality was lower among embryos exposed to 4 mg/L powdered rotenone for 12 h at 7 DPF (mean, 66%; 95% CL, 56.4 and 75%). Exposures to 2 mg/L powdered rotenone for 12 h did not increase embryo mortality at either developmental stage. A weak three-way interaction among treatment, exposure duration, and developmental stage was indicated (ANOVA: $F_{2, 24} = 2.69$, $P = 0.08$). A two-way interaction between treatment and exposure duration (ANOVA: $F_{2, 24} = 18.94$, $P < 0.001$) indicated that as powdered rotenone concentration increased embryo mortality was greater at 12-h exposure durations than after 4-h exposure durations. A two-way interaction between treatment and developmental stage (ANOVA: $F_{2, 24} = 3.39$, $P = 0.05$) indicated that increased powdered rotenone concentration resulted in greater embryo mortality following exposures at the late developmental stage than after exposures at the early developmental stage. A two-way interaction between developmental stage and exposure duration (ANOVA: $F_{1, 24} = 7.12$, $P = 0.01$) indicated that longer exposure durations resulted in higher embryo mortality following exposures at the late developmental stage than after exposures at the early developmental stage.

Powdered rotenone exposures increased Rainbow Trout embryo mortality in 4 and 12 h treatments (Figure 6). Embryo mortality was highest following 4 mg/L

powdered rotenone exposures for 12 h at both 5 DPF (mean, 66%; 95% CL, 56.7 and 75.3%) and 16 DPF (mean, 85%; 95% CL, 77.6 and 91.7%). Powdered rotenone exposures of 2 mg/L for 12 h increased embryo mortality at 5 DPF (mean, 47%; 95% CL, 37.2 and 56.8%) but not at 16 DPF (mean, 33%; 95% CL, 23.5 and 41.9%). Exposures of 4 mg/L for 4 h at 5 DPF resulted in increased embryo mortality (mean, 47%; 95% CL, 36.9 and 56.4%). No other 4-h exposure increased embryo mortality. A three-way interaction among treatment, exposure duration, and developmental stage (ANOVA: $F_{2, 24} = 14.2$, $P < 0.001$) indicated that as powdered rotenone concentration increased the influence of exposure duration on embryo mortality was greater at the late developmental stage than at the early developmental stage.

Rotenone Solvents. Rotenone solvents NMP (Figure 7) and DGEE (Figure 8) did not increase mortality among Rainbow Trout embryos. Mortality among NMP treatments was not detectably different from controls at any combination of exposure duration and developmental stage; therefore, no evidence supported a NMP treatment effect (ANOVA: $F_{2, 24} = 0.7$, $P = 0.5$). A moderate DGEE treatment effect on embryo mortality was evident (ANOVA: $F_{2, 24} = 4.28$, $P = 0.03$). However, Tukey-Kramer pairwise comparisons indicated treatment mortality was not detectably different from controls.

Sodium Chloride. Exposure to sodium chloride treatments did not result in increased mortality in Lake Trout embryos (Figure 9; ANOVA: $F_{2, 58} = 0.47$, $P = 0.63$).

Calcium Carbonate. Calcium carbonate treatments did not result in detectably different mortalities among Rainbow Trout embryos at the concentrations selected

(Figure 10). No evidence supported a calcium carbonate treatment effect on embryo mortality (ANOVA: $F_{2, 24} = 1.17$, $P = 0.33$). Higher concentrations in Lake Trout embryo treatments did not increase mortality compared to controls (Figure 11). No evidence supported a calcium carbonate treatment effect on Lake Trout embryo mortality (ANOVA: $F_{2, 24} = 0.29$, $P = 0.75$). Calcium carbonate treatments did not result in elevated levels of mortality among larval Lake Trout (Figure 12; ANOVA: $F_{2, 30} = 2.45$, $P = 0.1$).

Gelatin. Exposure to gelatin increased mortality of Rainbow Trout embryos at 5 DPF but not at 16 DPF (Figure 13). Exposure to 8,166 mg/L gelatin for 12 h at 5 DPF resulted in a mean percent mortality of 40% (95% CL, 30.7 and 49.9%) compared to 19% (95% CL, 11.6 and 27.1%) among control embryos. No other gelatin treatment at 5 DPF resulted in increased embryo mortality. A two-way interaction between treatment and developmental stage (ANOVA: $F_{2, 24} = 7.11$, $P = 0.003$) indicated that increased gelatin concentration resulted in greater embryo mortality following exposure at the early developmental stage than at the late developmental stage. Exposure to gelatin did not increase Lake Trout embryo mortality (Figure 14; ANOVA: $F_{2, 24} = 0.04$, $P = 0.96$) or larval mortality (Figure 15; ANOVA: $F_{2, 30} = 0.87$, $P = 0.43$).

Carcass and Carcass Analogs

Whole Carcass. Exposure to a whole carcass increased Rainbow Trout embryo mortality at the 0.8 L/min flow rate, but not at the 3.8 and 7.6 L/min flow rates (Figure 16). Exposure to a whole carcass at 0.8 L/min resulted in a mean percent mortality of

42% (95% CL, 32.6 and 52%) among treatment embryos compared to 19% (95% CL, 11.6 and 27%) among control embryos. Exposure to a whole carcass at 3.8 or 7.6 L/min flow rates did not increase embryo mortality. A two-way interaction between treatment and flow (ANOVA: $F_{2,12} = 3.86$, $P = 0.051$) indicated that exposure to a whole carcass resulted in higher embryo mortality at low flow rates than at high flow rates.

Low flow rates in whole carcass experiments with Lake Trout embryos resulted in high mortality among controls (Figure 17). Flow rates of 0.2 and 0.4 L/min resulted in 100% mortality in both treatment and control embryos. However, time to reach 100% mortality differed between treatments and controls. Treatment embryos reached 100% mortality 6 days after initial exposure to carcass material and control embryos reached 100% mortality on day 39 at the 0.2 L/min flow rate (Figure 18). Similarly, treatment embryos at the 0.4 L/min flow rate reached 100% mortality 14 days after initial exposure to carcass material whereas control embryos reached 100% mortality on day 46 (Figure 19). Dissolved oxygen concentrations in 0.2 and 0.4 L/min carcass treatments were reduced compared to controls. Mean dissolved oxygen concentrations were 1.6 mg/L in 0.2 L/min treatments and 4.7 mg/L in 0.4 L/min treatments. Mean control dissolved oxygen concentrations were 8.7 and 9.0 mg/L in 0.2 and 0.4 L/min carcass treatments, respectively. Mean percent mortality of treatment embryos at the 0.8 L/min flow rate was 70% (95% CL, 61 and 78.9%) and was detectably different (Figure 17; $t = -2.88$, $df = 4$, $P = 0.04$) from the mean percent control mortality of 56% (95% CL, 46.9 and 66.4%). Mean dissolved oxygen concentrations remained high for the duration of the experiment in both the 0.8 L/min treatment (10.6 mg/L) and control (11.0 mg/L). Water temperatures

increased within incubators as a result of lowering flow rates (Figure 20). Mean daily temperatures in 0.2, 0.4, and 0.8 L/min treatments were 10.9, 10.2, and 9.9 °C, respectively.

Static Carcass. Exposure to carcass material in static experiments resulted in 100% mortality among Rainbow Trout treatment embryos (Figure 21). Treatment mortality occurred rapidly, reaching 100% by day nine of the experiment and was detectably different from control mortality ($t = -6.93$, $df = 4$, $P = 0.002$). Mean percent mortality of control embryos was 20% (95% CI, 12.2 – 27.8).

Static carcass experiments with Lake Trout embryos resulted in 100% mortality among control and treatment embryos (Figure 21). However, time to reach 100% mortality differed between treatments and controls (Figure 22). Treatment embryos reached 100% mortality 24 days after initial exposure to carcass material and control embryos reached 100% mortality on day 40. Bubblers maintained dissolved oxygen concentrations in these experiments as expected; mean dissolved oxygen concentration was 9.9 mg/L among treatment exposure boxes and 10.4 mg/L among control exposure boxes.

Ground Carcass. Exposure to ground carcass material increased Rainbow Trout embryo mortality at high biomass levels (Figure 23). Treatment mortality was not detectably different among biomass levels (ANOVA: $F_{3, 36} = 0.59$, $P = 0.62$). Mean treatment embryo mortality ranged from 34% (95% CL, 24.5 and 42.8%) to 39% (95% CL, 29.1 and 48.1%). Mean control embryo mortality ranged from 21% (95% CL, 13.4

and 29.4) to 39% (95% CL, 29.2 and 48.2) and decreased with descending position within the vertical flow incubator (ANOVA: $F_{3, 36} = 11.85$, $P < 0.001$). Differences in control mortality may be attributable to decreasing light intensity among descending trays within the vertical flow incubator. A two-way interaction between treatment and biomass (ANOVA: $F_{3, 72} = 4.14$, $P = 0.009$) indicated that the effect of ground carcass exposure on embryo mortality was greater at high biomass levels than at low biomass levels.

Ground carcass material did not influence the water quality parameters monitored in laboratory experiments. Dissolved oxygen concentrations remained high for the duration of the experiment at all biomass levels (Figure 24). Mean dissolved oxygen concentrations ranged from 7.0 to 7.5 mg/L among treatments. Ground carcass material did not influence pH (Figure 25,) or ammonia concentrations (Figure 26). Hydrogen sulfide briefly increased in ground carcass exposures of 10 kg/m² and higher on day three of the experiment (Figure 27) but returned to levels similar to controls for the remainder of the experiment on day four.

Carcass Analogs. Exposure to Rainbow Trout carcass analog pellets increased Rainbow Trout embryo mortality compared to controls in 10, 14, and 19 kg/m² biomass treatments, but not in 5 kg/m² biomass treatments (Figure 23). Almost all embryos exposed to biomass treatments of 14 kg/m² (mean 99%; 95% CL, 99.3 and 100%) and 19 kg/m² (mean, 98%; 95% CL, 95.9 and 100%) died. Mean percent mortality was 49% (95% CL, 38.7 and 58.3%) among embryos in the 5 kg/m² biomass treatment and 53% (95% CL, 42.8 and 62.4%) in the 10 kg/m². Control embryo mortality ranged from 21% (95% CL, 13.4 and 29.4%) to 39% (95% CL, 29.2 and 48.2%). A two-way interaction

between treatment and biomass (ANOVA: $F_{3, 72} = 66.47$, $P < 0.001$) indicated that embryo mortality was greater at high carcass analog biomass exposures than at low biomass exposures.

Rainbow Trout carcass analog pellets influenced water quality parameters. Dissolved oxygen concentrations decreased with compounding carcass analog biomass (Figure 24). Mean dissolved oxygen concentrations were 6.5 mg/L in the 5 kg/m² treatment, 5.1 mg/L in the 10 kg/m² treatment, 3.9 in the 14 kg/m² treatment, and 3.7 mg/L in the 19 kg/m² treatment. Mean control dissolved oxygen concentrations ranged from 7.9 to 8.0 mg/L. Rainbow Trout carcass analogs did not influence pH (Figure 25). Ammonia concentrations increased with increasing carcass analog biomass (Figure 26). Mean ammonia concentrations were 0.21 mg/L in the 5 kg/m² treatment, 0.45 mg/L in the 10 kg/m² treatment, 0.64 mg/L in the 14 kg/m² treatment, and 0.78 mg/L in the 19 kg/m² treatment. Mean control ammonia concentrations range from 0.03 to 0.04 mg/L. Concentrations of hydrogen sulfide were elevated in the 14 and 19 kg/m² Rainbow Trout carcass analog treatments (Figure 27) from day one through day nine of the experiment. Hydrogen sulfide concentrations returned to levels similar to controls on day ten and remained low for the remainder of the experiment.

Exposure to plant-based carcass analog pellets increased Rainbow Trout embryo mortality compared to controls in 10, 14, and 19 kg/m² biomass treatments, but not in 5 kg/m² biomass treatments (Figure 23). Exposure to plant-based carcass analogs in biomass treatments of 14 and 19 kg/m² resulted in 100% mortality. Mean percent mortality was 84% (95% CL, 76.6 and 91%) among embryos exposed to 10 kg/m² pellet

biomass and 39% (95% CL, 29.3 and 48.5%) among embryos exposed to 5 kg/m² pellet biomass. A two-way interaction between treatment and biomass (ANOVA: $F_{3,72} = 95.99$, $P < 0.001$) indicated that a difference in embryo mortality between treatments and controls existed at high carcass analog pellet biomass exposures but not at low biomass exposures.

Plant-based carcass analog pellets influenced water quality parameters. Dissolved oxygen concentrations decreased with compounding carcass analog biomass (Figure 24) similar to Rainbow Trout carcass analogs (Table 2). Exposure to plant-based carcass analogs did not influence pH (Figure 25). Ammonia concentrations increased in plant-based carcass analog exposures (Figure 26; Table 2). Hydrogen sulfide briefly increased in plant-based carcass analog exposures of 10, 14, and 19 kg/m² on August 5 (Figure 27) but returned to levels similar to controls for the remainder of the experiment on August 6.

Sedimentation. Sediment depth affected embryo mortality in short-term laboratory sedimentation experiments (Figure 28). Mean percent mortality of embryos in short-term experiments treated with 10, 15, and 20 mm sediment depths were 72% (95% CL, 63.2 and 80.8%), 72% (95% CL, 63.2 and 80.8%), and 78% (95% CL, 69.9 and 86.1%), respectively. Mean percent mortality of embryos in the 5 mm sediment depth treatment was 38% (95% CL, 28.5 and 47.5%) and control mortality was 46% (95% CL, 36.2 and 55.8%). Embryo mortality in long-term experiments was higher among all sediment depths than mortality in short-term experiments (Figure 28). Treatments of 10, 15, and 20 mm sediment depths resulted in 100% embryo mortality. Mean percent mortality of embryos in the 5 mm sediment treatment was 86% (95% CL, 79.2 and

92.8%) and mean percent mortality of controls was 90% (95% CL, 84.1 and 95.9%).

Sediment depth (ANOVA: $F_{4, 40} = 4.89$, $P = 0.002$) and duration of sediment cover (ANOVA: $F_{1, 40} = 46.76$, $P < 0.001$) affected embryo mortality. No evidence supported a two-way interaction between depth and duration on embryo mortality (ANOVA: $F_{4, 40} = 1.04$, $P = 0.4$).

Field Trials

Ground Carcass. The application of ground carcass material to experimental sites at Thomas Bank increased short-term Lake Trout embryo mortality (Figure 29). Mean percent mortalities of embryos at the substrate surface were 18% (95% CL, 7.4 and 28.6%) among controls, 64% (95% CL, 51.1 and 77.7%) among light treatment embryos, and 100% among medium and heavy treatment embryos. Mean percent mortalities at 20 cm in the substrate were 12% (95% CL, 2.5 and 20.4%) among controls, 99 % (95% CL, 97.1 and 100%) among light treatment embryos, and 100% among medium and heavy treatment embryos. A two-way interaction between treatment and depth (ANOVA: $F_{3, 64} = 9.19$, $P < 0.001$) indicated that the effect of increasing ground carcass biomass on embryo mortality was greater at 20 cm within the substrate than at the substrate surface.

Long-term effects of ground carcass application were similar to short-term effects (Figure 30). Mean percent mortalities of embryos at the substrate surface were 63% (95% CL, 49.3 and 76.1%) among controls, 85% (95% CL, 75 and 94.8%) among light treatment embryos, and 100% among medium and heavy treatment embryos. Mean percent mortalities at 20 cm in the substrate were 29% (95% CL, 16.3 and 41.5%) among controls and 100% among light, medium, and heavy treatment embryos. A two-way

interaction between treatment and depth (ANOVA: $F_{3,64} = 35.19$, $P < 0.001$) indicated that the effect of increasing ground carcass biomass on embryo mortality was greater at 20 cm within the substrate than at the substrate surface.

Ground carcass deposition at Thomas Bank reduced dissolved oxygen concentrations at the substrate surface in medium and heavy biomass treatment sites, but not in control and light biomass treatment sites (Figure 31). Dissolved oxygen concentrations declined following ground carcass deposition on September 27, 2017, in medium and heavy treatment sites. The minimum mean daily dissolved oxygen concentration was 0.1 mg/L in medium treatment sites and 0.06 mg/L in heavy treatment sites, both occurring on October 5, 2017. Dissolved oxygen concentrations began increasing on October 6, 2017, in medium and heavy treatment sites and subsequently remained high for the duration of the experiment (Figure 32).

No detectable difference was found in the change in biofilm dry biomass between treatment and control sites at Thomas Bank following ground carcass deposition (Table 3). Among the three comparisons between the treatments and control, the maximum difference in the mean change in biofilm dry biomass was 0.000185 g/m² between light treatment sites and control sites and no evidence suggested this difference was > 0 (paired t -test, $df = 2$, t -stat = 0.33, $P = 0.386$; Table 3).

Deep-water ground carcass deposition at Olson Reef did not influence water quality (Figure 33). About 2,156 kg of ground carcass material was deposited at Olson Reef over a 6-d period from October 2, 2017 to October 7, 2017. Dissolved oxygen concentrations remained high for the duration of the experiment. The lowest mean daily

dissolved oxygen concentration of 6.0 mg/L occurred on October 16, 2017, about nine days following the last application of carcass material.

Sedimentation. The deposition of sediment did not increase Lake Trout embryo mortality in short-term incubators at Carrington Island experimental sites (Figure 34). Mean percent mortality at the substrate surface was 45% (95% CL, 31.5 and 59.3%) among control embryos and 43% (95% CL, 29.2 and 56.6%) among treatment embryos. Mean percent mortality at 20 cm in the substrate was 20% (95% CL, 8.6 and 30.6%) among control embryos and 29% (95% CL, 16.7 and 42.0%) among treatment embryos. No evidence indicated embryo mortality differed between treatment and control (ANOVA: $F_{1, 32} = 0.54$, $P = 0.47$).

Sediment deposition increased Lake Trout embryo mortality in long-term incubators at Carrington Island (Figure 35). Mean percent mortality at the substrate surface was 70% (95% CL, 57.5 and 83%) among control embryos and 97% (95% CL, 92.9 and 100%) among treatment embryos. Mean percent mortality at 20 cm in the substrate was 50% (95% CL, 35.6 and 63.5%) among control embryos and 100% among treatment embryos. A two-way interaction between treatment and depth (ANOVA: $F_{1, 32} = 39.1$, $P < 0.001$) indicated that the effect of sediment deposition on embryo mortality was greater at 20 cm within the substrate than at the substrate surface.

Sediment deposition at Carrington Island reduced dissolved oxygen concentrations at the substrate surface within treatment sites (Figure 36). Dissolved oxygen concentrations declined following sediment deposition on October 5, 2017. The minimum mean daily dissolved oxygen concentration during short-term experiments was

1.38 mg/L occurring on October 6, 2017. Dissolved oxygen concentrations in treatment sites increased to levels similar to control sites from October 20 through December 8 (Figure 37). Dissolved oxygen concentrations within treatment sites decreased again on December 9 and remained below that of controls for the remainder of the experiment (Figure 37). The minimum mean daily dissolved oxygen concentration during long-term experiments was 0.04 mg/L occurring on February 11, 2018.

Discussion

Liquid rotenone, powdered rotenone, carcasses, carcass analogs, and sediment deposition caused mortality of Lake Trout embryos. However, these suppression methods differed in their effectiveness, rate at which mortality was achieved, and ease of application. Ground carcass exposure was highly effective, caused mortality rapidly, and was easy to apply in the field. Similarly, carcass analogs caused high embryo mortality rapidly in laboratory experiments but lacked field evaluation. Liquid and powdered rotenone exposures were most effective at late developmental stages, thereby limiting their field applicability. Sediment deposition was effective but labor intensive and caused delayed mortality. These differences, as well as Lake Trout spawning site characteristics such as depth, contour, fetch, substrate size, interstitial depth, isolation, and non-target organisms ultimately determine which embryo suppression method will be most applicable in a given situation.

Both liquid and powdered rotenone caused high embryo mortality in laboratory experiments. High concentrations, long exposure durations, and exposure during late

developmental stages increased the effectiveness of both formulations. High concentrations and long exposures commonly increase mortality in toxicological trials (Olson and Marking 1975; Srivastava et al. 2004), and late developmental stages of Lake Trout embryos are particularly sensitive to environmental contaminants (Allen et al. 2005) because trans-chorionic transport of toxicants is driven by diffusion (Mäenpää et al. 2004). The chorion provides protection by binding noxious chemicals early in development, but bioaccumulation increases in late developmental stages as binding sites become saturated (Finn 2007). Additionally, the permeability of the chorion changes during embryonic development becoming increasingly porous as hatch approaches (Suga 1963). Therefore, I recommend 12-h exposures of 4 mg/L rotenone at late developmental stages (>260 degree days) to maximize embryo mortality.

Concentrations of liquid and powdered rotenone required to cause high Lake Trout embryo mortality (4.0 mg/L) were higher than those reported by Olson and Marking (1975), probably because my exposures were acute (4 or 12 h to better mimic realistic application scenarios) whereas theirs were chronic. Concentrations as low as 0.250 mg/L of liquid rotenone caused 50% mortality (LC50) of Lake Trout embryos during 192-h exposures (Olson and Marking 1975). My results more closely resembled the 96-h LC50s of Rainbow Trout embryos, which ranged from 2.50 to 5.60 mg/L (Marking and Bills 1976). The effects of dose and exposure duration compounded mortality in my experiments. However, lower doses could cause similar embryo mortality if longer exposure durations could be achieved (Olson and Marking 1975).

The rotenone emulsifiers NMP and DGEE did not increase embryo mortality indicating that mortality in rotenone treatments was a result of exposure to rotenone and not to solvent components present in piscicidal formulations of rotenone. The concentrations of NMP and DGEE in piscicidal formulations of rotenone were probably too low to cause embryo mortality. The 96-h LC50 of NMP to fish is 4,000 mg/L (as reported on its material safety data sheet, Sigma-Aldrich). Similarly, the 96-h LC50 for DGEE is 9,650 mg/L (as reported on its material safety data sheet, Sigma-Aldrich). These concentrations are several orders of magnitude greater than found in liquid rotenone.

Large-scale field application of liquid or powdered rotenone may be difficult. Delivery and retention of rotenone to interstices of spawning substrates at the required concentrations and exposure durations in a large-lake setting may be affected by embryo depth, rotenone dilution, and water movement. Lake Trout embryos can penetrate interstices in cobble substrates to at least 1 m (Marsden and Tobi 2014) and therefore a weighted pelletized form of rotenone would probably be required to reach such depths. Slow release of rotenone would be required to ensure exposure duration but dilution and currents could make application impossible in some places. Lake Trout typically deposit embryos in areas where wave-generated turbulence keeps interstices free of sediment and maintains dissolved oxygen concentrations (Fitzsimons and Marsden 2014). Hydrology experiments conducted at a Lake Trout spawning site in Yellowstone Lake confirmed that currents rapidly dispersed injected sodium chloride within interstices (Thomas 2017). Additionally, rotenone may affect non-target organisms such as Yellowstone Cutthroat Trout and aquatic macroinvertebrates (Chandler and Marking 1982; Finlayson et al.

2010). Finally, targeting late developmental stages of Lake Trout embryos in Yellowstone Lake may be limited by the logistical constraints of working in the interior of Yellowstone National Park during winter.

Sodium chloride, calcium carbonate, and gelatin did not increase Lake Trout embryo mortality because of short exposure durations, low concentrations, or both. Concentrations of sodium chloride were based on salinities that reduced hatching success of embryos of multiple fish species in chronic exposures (Koel and Peterka 1995), but were probably too low to induce mortality in acute exposures. Episodic 24-h exposures of sodium chloride up to 10,000 mg/L did not decrease survival of Atlantic Salmon embryos (Mahrosh et al. 2014). Likewise, I selected concentrations of calcium carbonate based on those implicated in reducing Lake Trout recruitment in Bear Lake, Utah and Idaho (Martinez et al. 2009), where exposure of embryos to calcium carbonate is chronic and includes precipitation on embryos. The geology of the Bear Lake region is dominated by limestone deposits that provide the calcium carbonate that enters the lake through weathering processes (Palacios et al. 2007). The geology of Yellowstone Lake differs and is dominated by andesite and rhyolite (Gresswell et al. 1997). An attempt to reproduce the water chemistry of Bear Lake at Lake Trout spawning sites in Yellowstone Lake would probably be labor intensive, requiring multiple applications of calcium carbonate, and ultimately may not be feasible. Gelatin slightly increased mortality among Rainbow Trout embryos in a single treatment and did not increase Lake Trout embryo mortality. Concentrations may have been too low to inhibit embryonic gas exchange or facilitate fungal growth. I decided that further evaluations of these benign chemical compounds

were not warranted because they did not cause sufficient embryo mortality in laboratory experiments.

Scheduling acute chemical treatments during critical stages in Lake Trout embryo development may increase total mortality. Short periods in early development when mortality is concentrated are considered critical stages (May 1974) and additional environmental stress during these stages may increase embryo mortality (Latif et al. 1999). Highest natural mortality of Lake Trout embryos occurred at 128 or 130 degree days during gastrulation and again at 360 and 368 degree days during organogenesis (Figure 2). My laboratory chemical treatments were performed prior to or between these critical stages at 64 or 70 degree days and 260 or 264 degree days. Effects of acute chemical treatments may therefore have been more pronounced had they coincided with the critical stages. However, scheduling treatments to occur simultaneously with critical stages of embryos in a natural setting could be difficult without exact knowledge of spawning times or if spawning is protracted.

Degraded water quality was probably the proximal cause for increased mortality of Lake Trout and Rainbow Trout embryos chronically exposed to carcass material in laboratory experiments. Decaying organic matter inhibits successful embryo incubation (Sly 1988; Marsden et al. 1995), whereas water exchange maintains water quality (Fitzsimmons and Marsden 2014). All embryos exposed to decaying carcass material at water turnover rates greater than 42 minutes died. Mortality was rapid, reaching 100% within as few as 6 days of exposure. Low flow rates also resulted in mortality among controls, but deaths occurred later than in the treatment incubators, and were probably

caused by chronic exposure to high water temperatures (Figure 20). High mortality is common in Lake Trout embryos developing at water temperatures above 10°C (Royce 1951; Carlson and Siefert 1974). Water entering incubators at low flow rates warmed appreciably in the incubators through contact with warm air.

Application of decaying fish carcasses increases Lake Trout embryo mortality by degrading water quality in natural settings. Experimental application of whole fish carcasses to Lake Trout spawning sites in Yellowstone Lake caused high embryo mortality, but wave action caused whole carcasses to drift off of treatment sites allowing water quality to recover (Thomas et al., in press). Therefore, ground carcass application was proposed to reduce drift by better infiltrating substrate interstices.

Field application of ground carcass material corroborated findings of my laboratory trials and whole-carcass field applications. Complete embryo mortality was obtained in the heavy biomass treatment (28 kg/m²) at both the substrate surface and 20 cm within the substrate, as in analogous whole carcass field experiments (Thomas et al., in press). Complete embryo mortality was also obtained in medium biomass treatments but mortality varied among light treatment sites. The variability derived mostly from a single site where embryo mortality was markedly lower than in other light treatment sites. Physical attributes of Lake Trout spawning sites such as slope and fetch may influence embryo mortality appreciably at light biomass application sites by maintaining adequate water quality through upwelling or wave action. Therefore, I recommend a minimum carcass biomass application rate of 14 kg/m² (medium treatment) for embryo suppression on Yellowstone Lake.

Carcass analog pellets, which heretofore had not been evaluated as an embryo suppression tool, may be more effective than fish carcasses because they are readily available, easy to apply, and more potent per unit weight than carcasses. Carcass analogs induce ecosystem responses similar to natural carcass deposition in salmonid spawning streams such as increased productivity at multiple trophic levels (Wipfli et al. 2004; Pearsons et al. 2007). However, if applied excessively, nutrients released from carcasses and carcass analogs can cause noxious algal blooms and depressed dissolved oxygen concentrations (Compton et al. 2006). Because both carcass and carcass analog exposures degraded water quality and caused embryo mortality in the laboratory, and because carcass deposition was effective in the field (Thomas et al., in press), I expect field applications of carcass analogs will be effective as well.

Exposure to carcass analogs caused higher embryo mortality than ground carcass material in similarly structured laboratory experiments, probably because nutrient densities of carcass analogs are higher than those of carcasses, which have a high water content. For example, water content of Brown Trout tissues was about 75%, (Jonsson and Jonsson 1998). Nutrient density in carcass analogs was about 5 times higher than in carcasses because of the differences in water content (Pearsons et al. 2007). My carcass analog pellets were dried in a pulse bed dryer to achieve a final water content of less than 8% as determined by microwave moisture analyzer and were therefore more potent than carcass material when applied at the same biomass level.

Hypoxia probably caused embryo mortality in carcass and carcass analog exposures. Decreasing dissolved oxygen concentrations in the environment reverses the

diffusion gradient that supplies developing embryos with oxygen and allows for the accumulation of waste products ultimately causing mortality if conditions persist (Rombough and Randall 1988). Dissolved oxygen requirements of salmonid embryos change as development progresses. Oxygen consumption rises as respiration rate and metabolic activity increase as the embryo grows (Mäenpää et al. 2004). Embryos are most sensitive to hypoxia between 100 and 200 degree days (Alderdice et al. 1958). All Lake Trout embryos incubated at 10°C died at dissolved oxygen concentrations below 3.4 mg/L (Garside 1959; Carlson and Siefert 1974); similarly, I observed rapid embryo mortality when dissolved oxygen concentrations fell below 3.4 mg/L in whole carcass and carcass analog laboratory experiments. Embryo mortality was low in ground carcass laboratory treatments in which mean dissolved oxygen concentrations were maintained above 7.0 mg/L. Dissolved oxygen concentrations almost immediately fell below lethal levels (Garside 1959; Carlson and Siefert 1974) after treatment in ground carcass field experiments and remained low for about 9 days in heavy and medium treatment sites where complete mortality was obtained, as in whole carcass experiments (Thomas et al., in press). Scattering of ground carcass material by wind and wave action coincided with the subsequent recovery of dissolved oxygen concentrations. Moreover, ground carcass deposition was not effective at the deep-water spawning site Olson Reef because drift of ground carcass material as it descended to the substrate apparently reduced densities to below those required to induce hypoxia.

Other water quality parameters did not appear to be associated with embryo mortality. Ground carcass and carcass analog treatments did not influence pH in

laboratory experiments. Concentrations of ammonia were elevated in carcass analog treatments, but were well below chronic lethal levels for Rainbow Trout embryos (Brinkman et al. 2009). Hydrogen sulfide concentrations may have influenced embryo mortality as even the very low levels I observed can reduce embryo survival (Adelman and Smith 1970; Smith and Oseid 1974). Hydrogen sulfide concentrations briefly reached similar levels in both ground carcass and carcass analog laboratory exposures but mortality was elevated only in the carcass analog treatments. Water quality parameters enhancing embryo mortality in field experiments such as carbon dioxide, hydrogen sulfide, ammonia, and fungal proliferation (Sly 1988; Thomas et al., in press) did not appear to influence embryo mortality in laboratory experiments, but water exchange in field experiments was probably lower than in incubators and may have allowed accumulation of such stressors.

Both carcass material and carcass analog pellets have advantages and limitations for embryo suppression in Yellowstone Lake. Lake Trout carcass material is available from ongoing gillnetting suppression in Yellowstone Lake, and its use in embryo suppression does not involve a net increase in nutrients to the system. However, treatment of the estimated 11.4 ha of Lake Trout spawning habitat in Yellowstone Lake (Phil Doepke, NPS fisheries biologist, personal communication) with carcass material is limited by spawning season catch rates. Current spawning season catch rates could not support treating 11.4 ha at the recommended 14 kg/m² rate for successful embryo suppression. Moreover, carcass deposition may be limited to protected shallow water sites where prescribed concentrations of carcass material can be achieved. Carcass

material can attract wildlife (Thomas et al., in press), and therefore its use may be limited to remote spawning sites where human-wildlife conflicts are improbable. In contrast, carcass analogs could alleviate reliance on gill net catches, increase handling efficiency, better infiltrate spawning substrate interstices, reduce wildlife conflicts, and potentially be more effective than carcass material at lower biomass levels. However, carcass analogs have not been evaluated in field applications. Cost may be prohibitive when manufacturing large quantities of carcass analogs. The estimated cost of producing 1,500 kg of carcass analog pellets was US\$26,000 (Wendy Sealey, U.S. Fish and Wildlife Service, personal communication). Treating 11.4 ha of spawning habitat at 14 kg/m² would require 1,641,600 kg of carcass analogs. However, if production of large quantities was desirable, the cost per kg would come down. The use of carcass analogs in Yellowstone Lake would represent a net increase in nutrients unless offset by removal of a complementary amount of Lake Trout carcass material. Both carcass and carcass analog deposition may affect littoral communities in Yellowstone Lake. Although no short-term increase in substrate biofilm has been detected following carcass deposition experiments (Thomas 2017), large-scale application of these methods may influence littoral community structure. Monitoring the biotic community of Lake Trout spawning sites treated with carcass or carcass analog material will be required to understand any potential negative effects this method may have if applied at larger spatial scales.

Large-scale field application of carcass analogs may be more efficient than application of ground carcass material because no processing is required during deposition and carcass analog pellets can be distributed mechanically. Pellets could be

applied by broadcast spreader mounted on the back of a boat, similar to how granulated lampricide and antimycin treatments are made (Dawson and Kolar 2003). Treatment areas could be measured with onboard GPS equipped chartplotter units to estimate densities of carcass analogs delivered. Additionally, aerial application could be achieved using fixed-wing aircraft or helicopters (Selbig 1974).

I confirmed that infilling of spawning substrate interstices with sediments containing over 20% sand (<2.00 mm) can be lethal to developing Lake Trout and other salmonid embryos (Hausle and Coble 1976, Sly 1988; Manny et al. 1995; Marsden et al. 1995; Argent and Flebbe 1999; Kock 2004). Sediment deposition was effective at causing high Lake Trout embryo mortality in field experiments at Carrington Island but appeared to take longer than with the other suppression methods evaluated. Sediment deposition at Carrington Island decreased dissolved oxygen concentrations in treatment sites which probably caused mortality among treatment embryos. Deposition of fine sediment can reduce permeability of spawning substrate interstices, thereby causing a reduction in dissolved oxygen delivery to developing embryos (Hausle and Coble 1976; Argent and Flebbe 1999) and reducing survival. Chronic exposure to low dissolved oxygen concentrations overwinter probably explains the differences between short and long-term embryo mortality I observed.

Large-scale sediment deposition was difficult using the Piranha Mini-Dredge (Model PS165E). Suction dredging was time and labor intensive. Dredging was frequently halted to clear aquatic vegetation clogging the suction head. A larger model dredge may be more effective and efficient in depositing large amounts of sediment on

Lake Trout spawning sites in Yellowstone Lake. Refinement of this technique could be achieved by determining the appropriate dredge for Lake Trout embryo suppression. Sediment deposition may be limited to shallow water spawning sites with suitable fine sediment located in close proximity. Sand and gravel were easiest to direct and apply whereas finer sediments such as silts and clays stayed in suspension and drifted off of treatment sites. As with carcass material, wind and wave action may limit sediment deposition effectiveness at sites exposed to long fetch. Wave-generated turbulence keeps spawning site interstices free of sediment and maintains dissolved oxygen concentrations (Fitzsimons and Marsden 2014).

Synergistic implementation of effective embryo suppression methods evaluated in my study alongside traditional suppression netting of older life stages in an Integrated Pest Management approach could hasten Lake Trout population decline (Sawyer 1980; Christie and Goddard 2003; Lechelt and Bajer 2016; Brown 2017; Thomas et al., in press). Embryo suppression may be particularly important in Yellowstone Lake, where Lake Trout pre-recruit survival is estimated to be 2.5 times higher than among Lake Trout populations within their native range (Syslo 2015). Furthermore, Lake Trout population growth rates are highly sensitive to age-0 survival rates (Ferreri et al. 1995; Syslo et al. 2011; Cox et al. 2013), and therefore successful embryo suppression could have population-level effects. Embryo suppression may also become increasingly cost-effective as adult Lake Trout density decreases in Yellowstone Lake and cost per Lake Trout harvested by traditional methods increases. Moreover, development, evaluation, and refinement of novel suppression techniques should continue in the future, especially

of techniques or compounds that could reduce dissolved oxygen concentrations within spawning substrates but that do not require nutrient additions, such as sodium sulfite and related salts (Westman and Hunter 1956; Dawson and Kolar 2003).

Tables

Table 1 Treatments evaluated in laboratory embryo suppression trials by species.

Species	Substance	Treatment	Duration	Stage	Flow (L/min)	Temp. (C°)
Lake Trout	<u>Chemical</u> Sodium chloride ^a	2,500 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	8
		5,000 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	8
	Calcium carbonate ^b	10,000 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	10
		50,000 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	10
	Gelatin ^b	500 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	10
		1,000 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	10
	Liquid rotenone ^a	2 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	8
		4 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	8
	Powdered rotenone ^b	2 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	10
		4 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	10
	<u>Carcass</u> Whole carcass ^b	600 g	Chronic	Cleavage-hatch	0.2	10
		600 g	Chronic	Cleavage-hatch	0.4	10
		600 g	Chronic	Cleavage-hatch	0.8	10
	Static carcass ^b	10 g	Chronic	Cleavage-hatch	-	10
Rainbow Trout	<u>Chemical</u> Calcium carbonate ^c	268 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
		1,000 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13

^aLake Trout embryos from Saratoga National Fish Hatchery, Saratoga, WY, USA (2015).

^bLake Trout embryos from Yellowstone Lake, Yellowstone National Park, WY, USA (2016).

^cRainbow Trout embryos from Ennis National Fish Hatchery, Ennis, MT, USA (2016).

Table 1 Continued.

Species	Substance	Treatment	Duration	Stage	Flow (L/min)	Temp. (C°)
Rainbow Trout	<u>Chemical</u> Gelatin ^c	5,833 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
		8,166 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
	N-Methyl-2-pyrrolidone ^c	0.2 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
		0.4 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
	Diethylene glycol monoethyl ether ^c	1.3 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
		2.6 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
	Liquid rotenone ^c	2 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
		4 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
	Powdered rotenone ^c	2 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
		4 mg/L	4 and 12 hours	Epiboly and organogenesis	7.6	13
	<u>Carcass & carcass analogs</u>					
	Whole carcass ^c	130 g	Chronic	Cleavage-hatch	1.9	13
		130 g	Chronic	Cleavage-hatch	3.8	13
		130 g	Chronic	Cleavage-hatch	7.6	13
	Static carcass ^c	10 g	Chronic	Cleavage-hatch	-	13
	Ground carcass ^c	5 kg/m ²	Chronic	Cleavage-hatch	2.5	13
		10 kg/m ²	Chronic	Cleavage-hatch	2.5	13
		14 kg/m ²	Chronic	Cleavage-hatch	2.5	13
		19 kg/m ²	Chronic	Cleavage-hatch	2.5	13
	Carcass analog pellet (fish) ^c	5 kg/m ²	Chronic	Cleavage-hatch	2.5	13
		10 kg/m ²	Chronic	Cleavage-hatch	2.5	13
		14 kg/m ²	Chronic	Cleavage-hatch	2.5	13
		19 kg/m ²	Chronic	Cleavage-hatch	2.5	13

^cRainbow Trout embryos from Ennis National Fish Hatchery, Ennis, MT, USA (2016, 2017).

Table 1 Continued.

Species	Substance	Treatment	Duration	Stage	Flow (L/min)	Temp. (C°)
Rainbow Trout	<u>Carcass & carcass analogs</u>					
	Carcass analog	5 kg/m ²	Chronic	Cleavage-hatch	2.5	13
	pellet (plant) ^c	10 kg/m ²	Chronic	Cleavage-hatch	2.5	13
		14 kg/m ²	Chronic	Cleavage-hatch	2.5	13
		19 kg/m ²	Chronic	Cleavage-hatch	2.5	13
	<u>Sediment</u>					
	Carrington Island ^c	5 mm	10 and 20 days	Cleavage-hatch	1	13
		10 mm	10 and 20 days	Cleavage-hatch	1	13
		15 mm	10 and 20 days	Cleavage-hatch	1	13
		20 mm	10 and 20 days	Cleavage-hatch	1	13

^cRainbow Trout embryos from Ennis National Fish Hatchery, Ennis, MT, USA (2017).

Table 2 Mean daily water quality parameter readings in 2017 carcass and carcass analog laboratory experiments.

Treatment	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Ammonia (mg/L)	Hydrogen sulfide (mg/L)
Control					
5 kg/m ²	12.4	8.0	8.0	0.03	0.01
10 kg/m ²	12.5	8.0	8.1	0.03	0.01
14 kg/m ²	12.5	8.0	8.1	0.04	0.01
19 kg/m ²	12.5	8.0	8.2	0.03	0.01
Ground Carcass					
5 kg/m ²	12.4	7.5	8.1	0.07	0.01
10 kg/m ²	12.5	7.2	8.1	0.11	0.02
14 kg/m ²	12.6	7.2	8.1	0.11	0.02
19 kg/m ²	12.6	7.0	8.1	0.16	0.04
RBT Carcass Analog					
5 kg/m ²	12.4	6.5	8.0	0.21	0.01
10 kg/m ²	12.5	5.1	7.9	0.45	0.03
14 kg/m ²	12.6	3.9	7.9	0.64	0.05
19 kg/m ²	12.6	3.7	7.9	0.78	0.06
Plant-based Carcass Analog					
5 kg/m ²	12.3	6.1	8.0	0.19	0.01
10 kg/m ²	12.5	4.9	7.9	0.53	0.03
14 kg/m ²	12.8	3.8	7.9	0.20	0.05
19 kg/m ²	12.9	3.1	7.9	0.40	0.11

Table 3 Difference in mean change in biofilm dry biomass (g/m²) before and after ground carcass deposition experiments between treatment sites (7 kg/m² light treatment, 14 kg/m² medium treatment, and 28 kg/m² treatment sites) and control sites at Thomas Bank, Yellowstone Lake, Yellowstone National Park in autumn 2017. A one-tailed paired *t*-test (alternative > 0) was used for statistical analysis.

Location	Comparison	Biofilm (g/m ²) difference	df	<i>t</i> -stat	<i>P</i> -value
Thomas Bank	light treatment–control	0.000185	2	0.776	0.260
Thomas Bank	medium treatment–control	0.000009	2	0.046	0.484
Thomas Bank	heavy treatment–control	0.000026	2	0.330	0.386

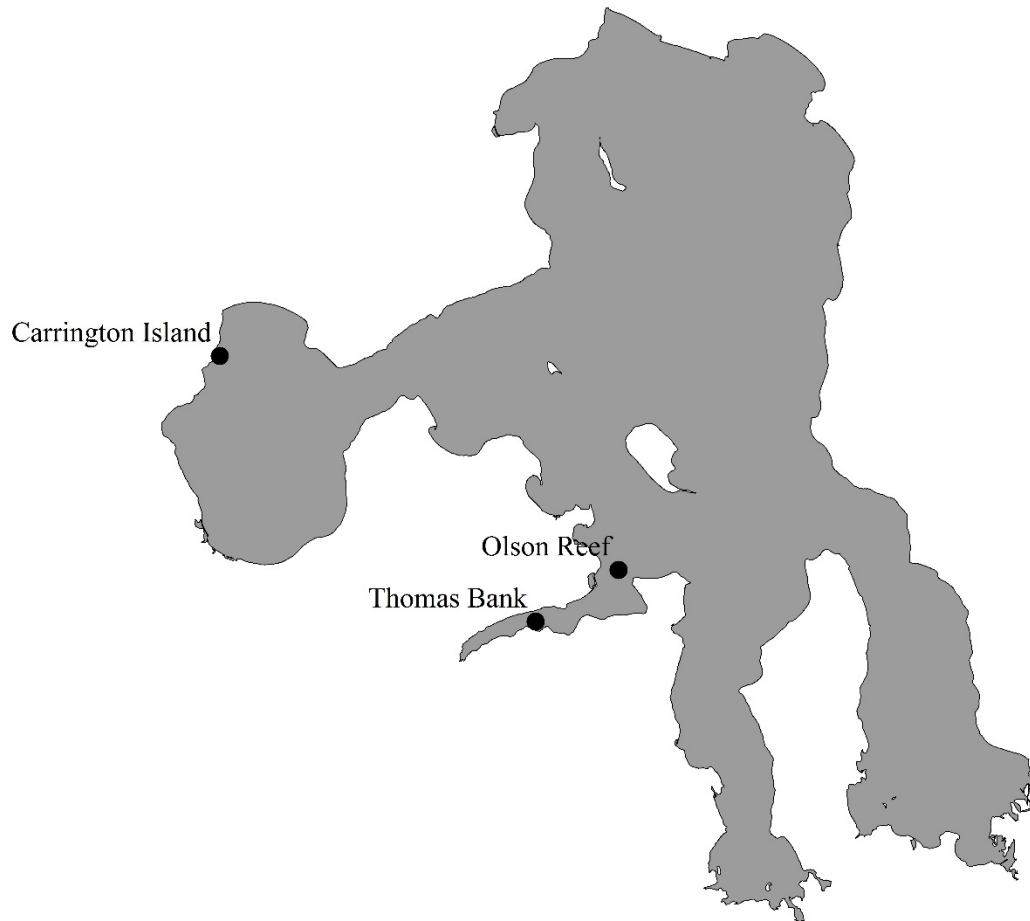
Figures

Figure 1. Locations of three confirmed Lake Trout spawning sites (delineated by black circles) in Yellowstone Lake, Yellowstone National Park, where *in situ* Lake Trout embryo suppression experiments were conducted. Ground carcass experiments were conducted at Thomas Bank and Olson Reef. Sedimentation experiments were conducted at Carrington Island.

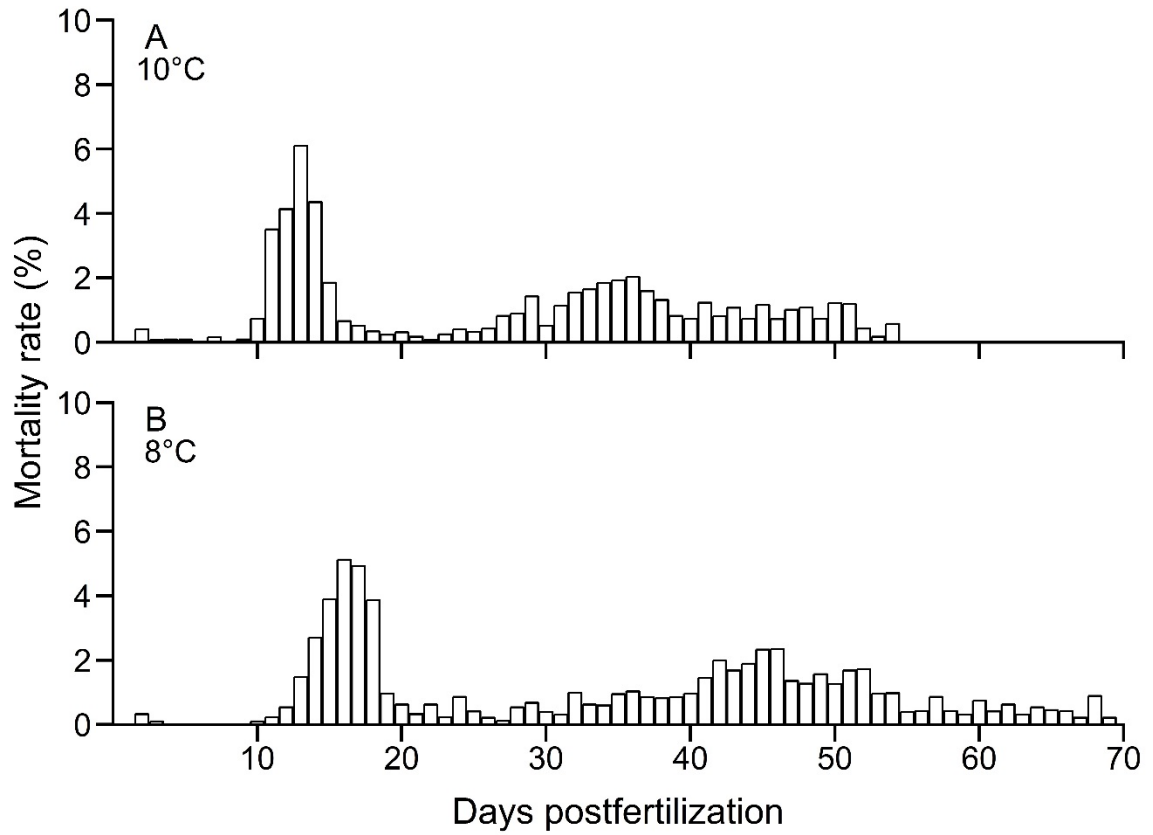


Figure 2. Mean daily percent mortality of Lake Trout embryos in embryonic developmental progression monitoring at 10°C (A) and 8°C (B) during incubation period.

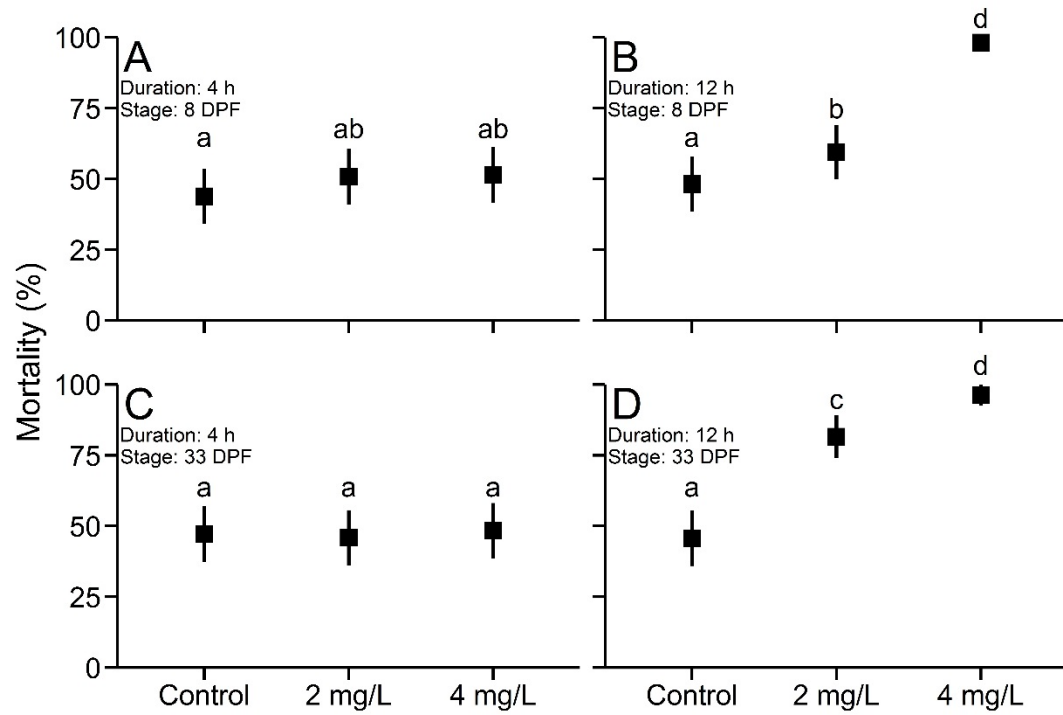


Figure 3. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) exposed to liquid rotenone for 4 h at 8 DPF (A), 12 h at 8 DPF (B), 4 h at 33 DPF (C), and 12 h at 33 DPF (D). Means denoted by the same letters were not statistically different.

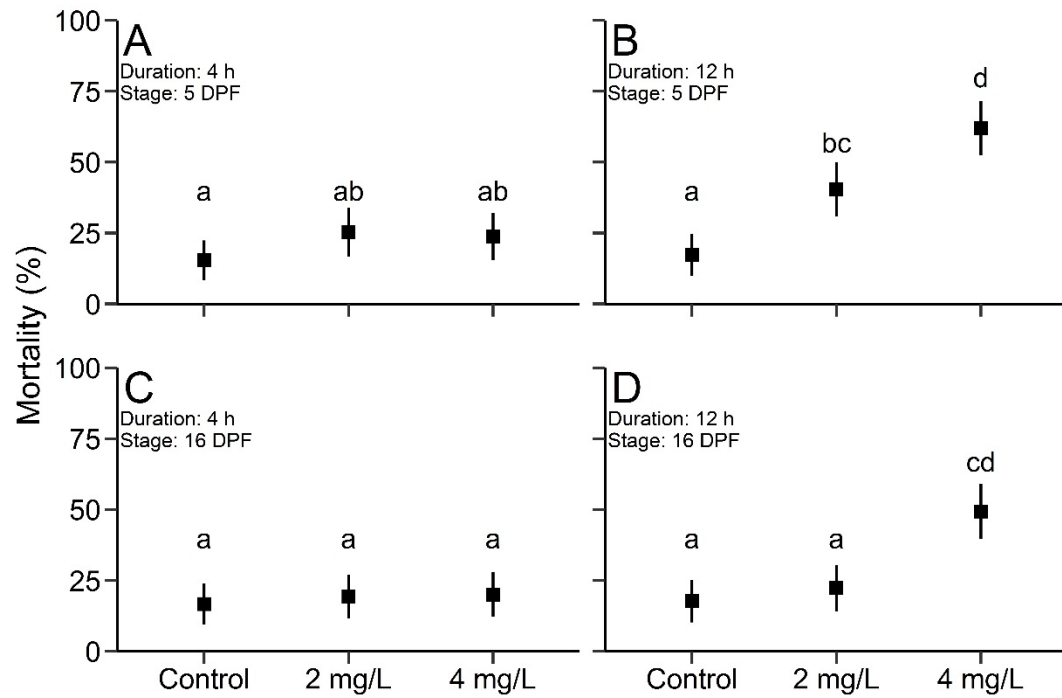


Figure 4. Percent mortality of Rainbow Trout embryos (mean $\pm$ 95% CL) exposed to liquid rotenone for 4 h at 5 DPF (A), 12 h at 5 DPF (B), 4 h at 16 DPF (C), and 12 h at 16 DPF (D). Means denoted by the same letters were not statistically different.

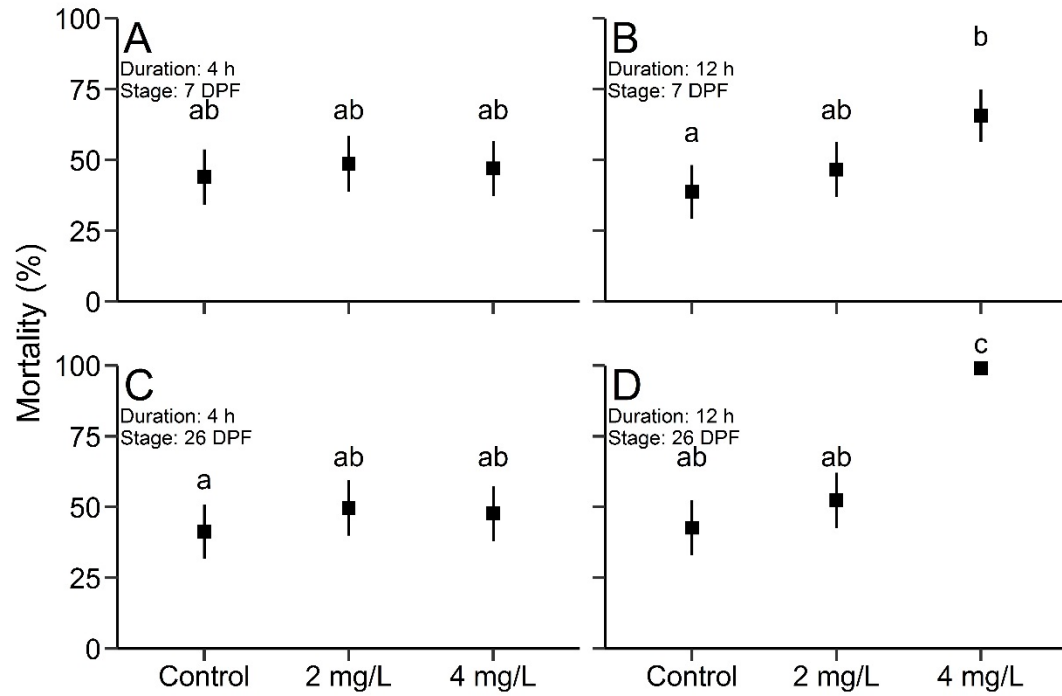


Figure 5. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) exposed to powdered rotenone for 4 h at 7 DPF (A), 12 h at 7 DPF (B), 4 h at 26 DPF (C), and 12 h at 26 DPF (D). Means denoted by the same letters were not statistically different.

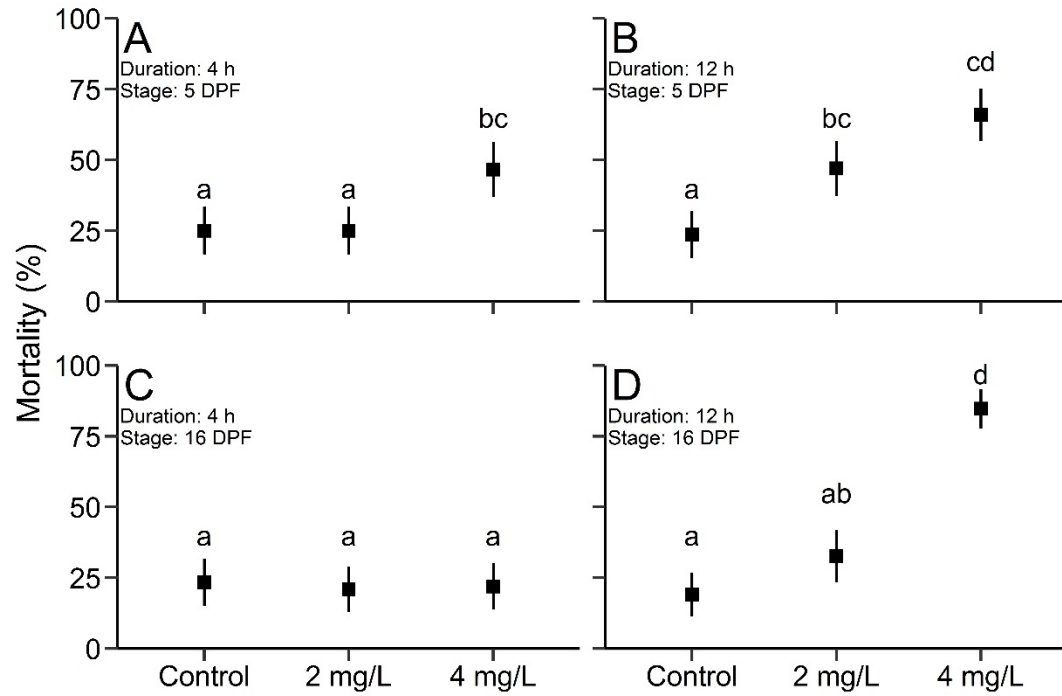


Figure 6. Percent mortality of Rainbow Trout embryos (mean $\pm$ 95% CL) exposed to powdered rotenone for 4 h at 5 DPF (A), 12 h at 5 DPF (B), 4 h at 16 DPF (C), and 12 h at 16 DPF (D). Means denoted by the same letters were not statistically different.

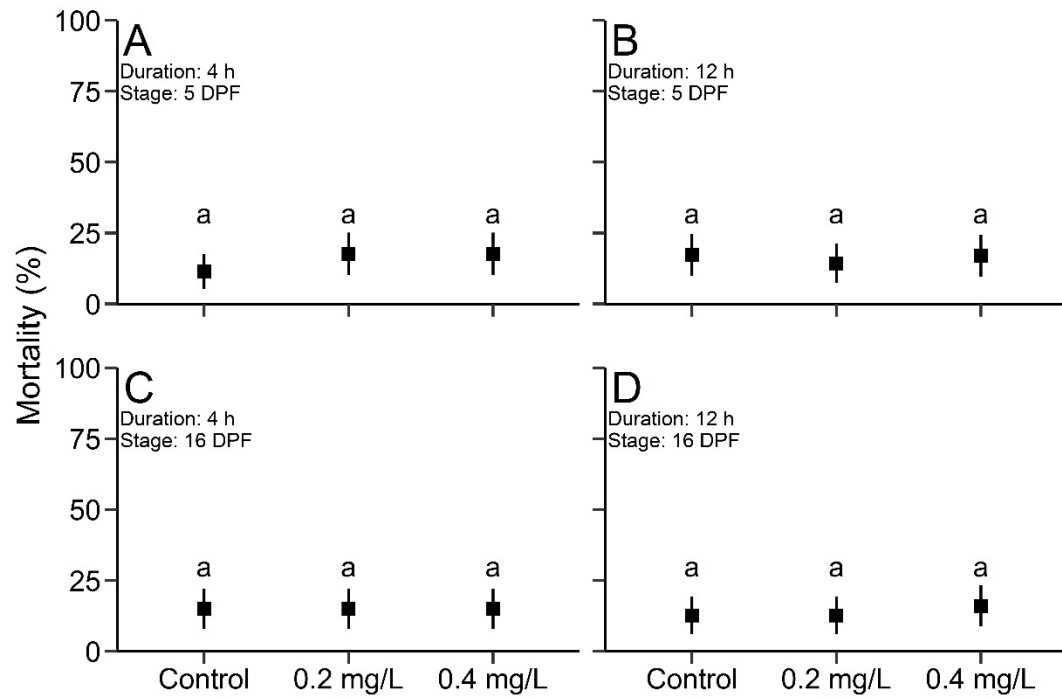


Figure 7. Percent mortality of Rainbow Trout embryos (mean $\pm$ 95% CL) exposed to N-methylpyrrolidone (NMP) for 4 h at 5 DPF (A), 12 h at 5 DPF (B), 4 h at 16 DPF (C), and 12 h at 16 DPF (D). Means denoted by the same letters were not statistically different.

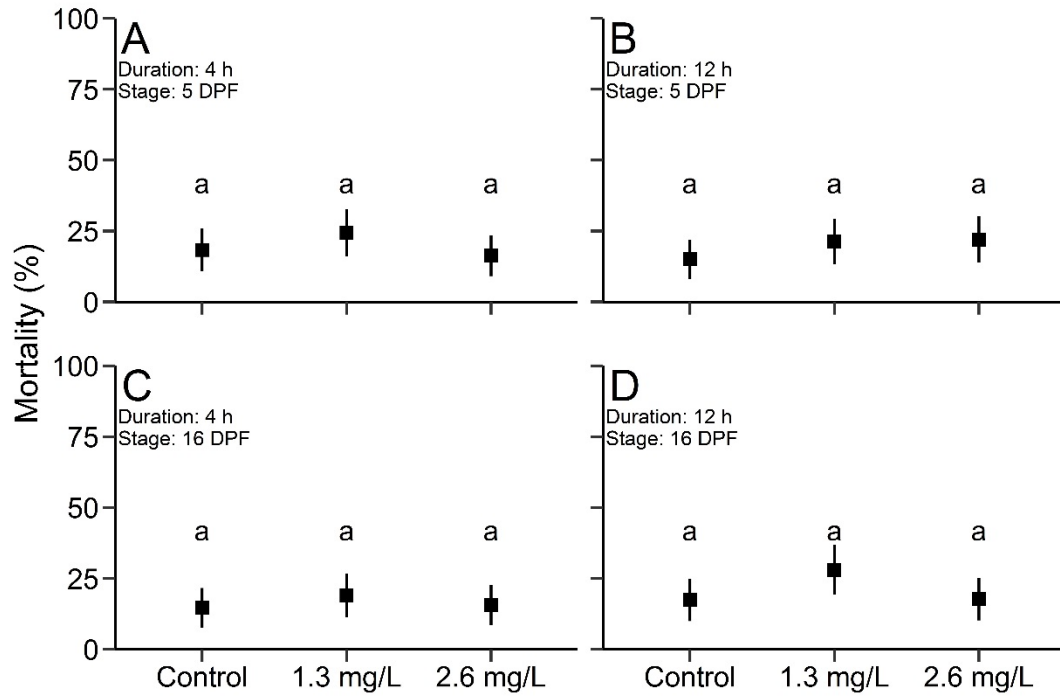


Figure 8. Percent mortality of Rainbow Trout embryos (mean $\pm$ 95% CL) exposed to diethylene glycol ethyl ether (DGEE) for 4 h at 5 DPF (A), 12 h at 5 DPF (B), 4 h at 16 DPF (C), and 12 h at 16 DPF (D). Means denoted by the same letters were not statistically different.

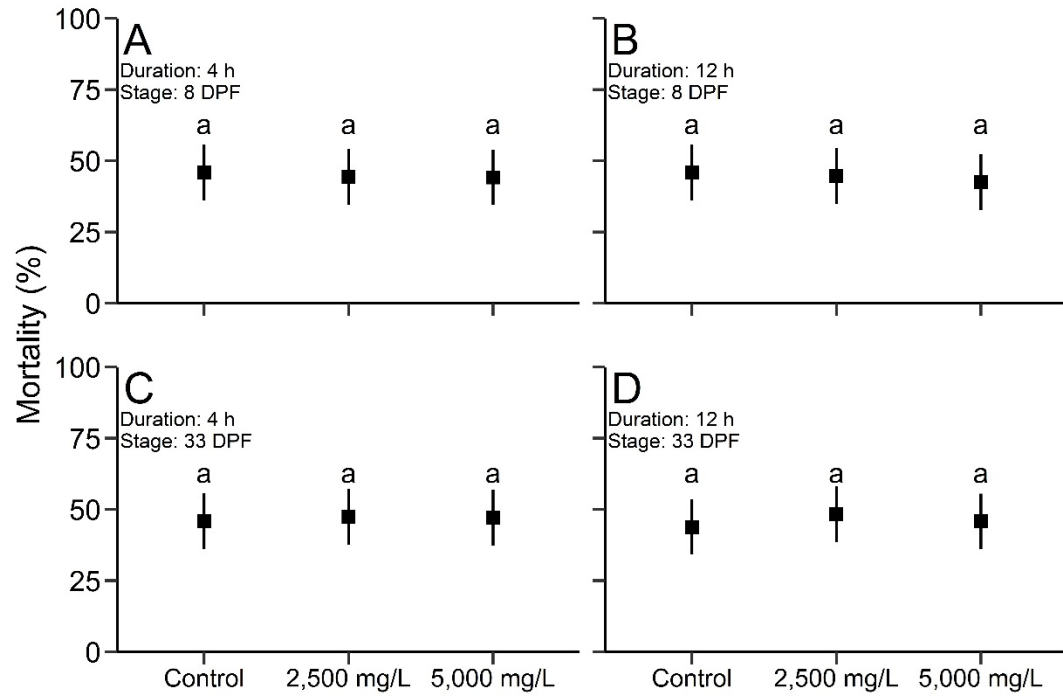


Figure 9. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) exposed to sodium chloride for 4 h at 8 DPF (A), 12 h at 8 DPF (B), 4 h at 33 DPF (C), and 12 h at 33 DPF (D). Means denoted by the same letters were not statistically different.

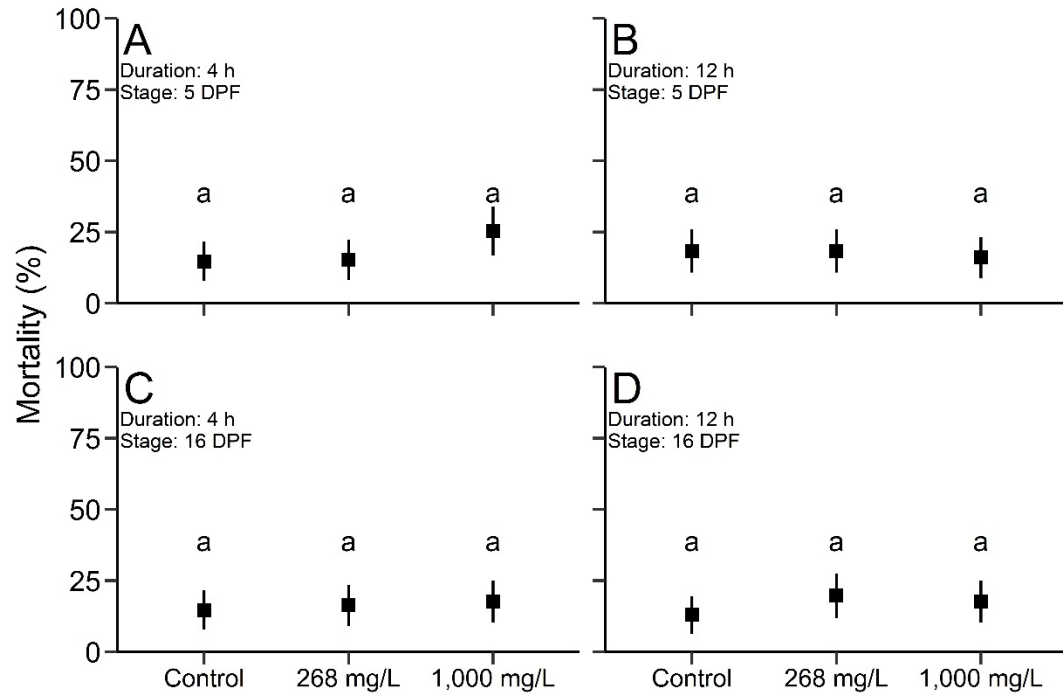


Figure 10. Percent mortality of Rainbow Trout embryos (mean $\pm$ 95% CL) exposed to calcium carbonate for 4 h at 5 DPF (A), 12 h at 5 DPF (B), 4 h at 16 DPF (C), and 12 h at 16 DPF (D). Means denoted by the same letters were not statistically different.

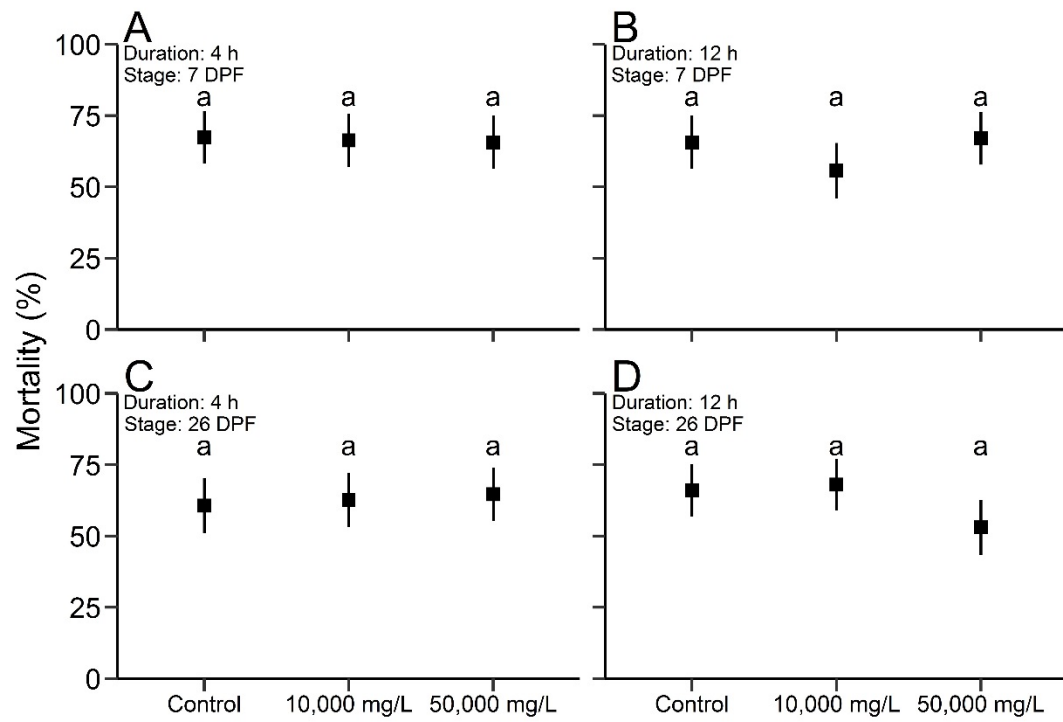


Figure 11. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) exposed to calcium carbonate for 4 h at 7 DPF (A), 12 h at 7 DPF (B), 4 h at 26 DPF (C), and 12 h at 26 DPF (D). Means denoted by the same letters were not statistically different.

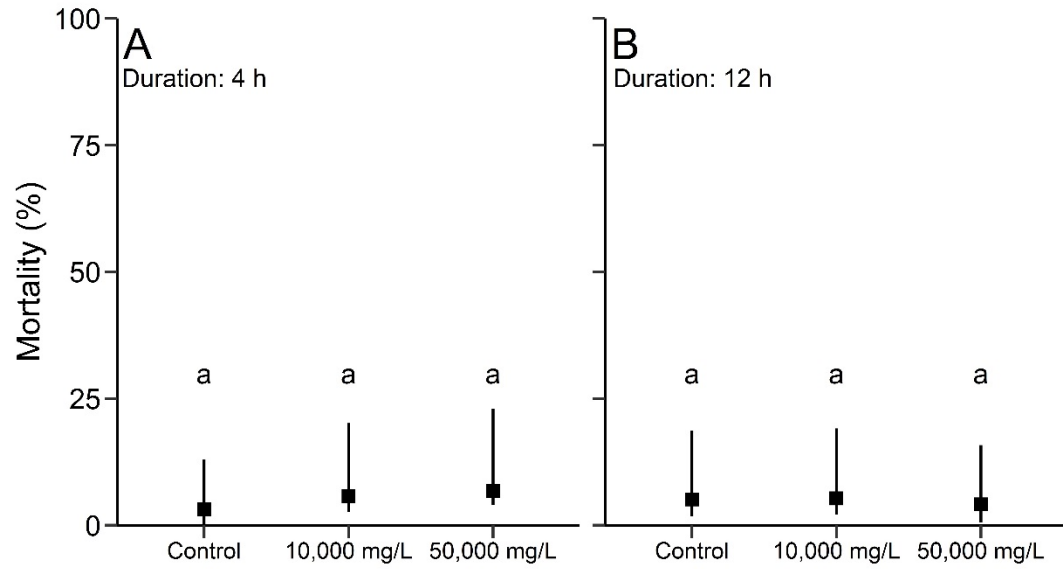


Figure 12. Percent mortality of Lake Trout larvae (mean $\pm$ 95% CL) exposed to calcium carbonate for 4 h (A), and 12 h (B). Means denoted by the same letters were not statistically different.

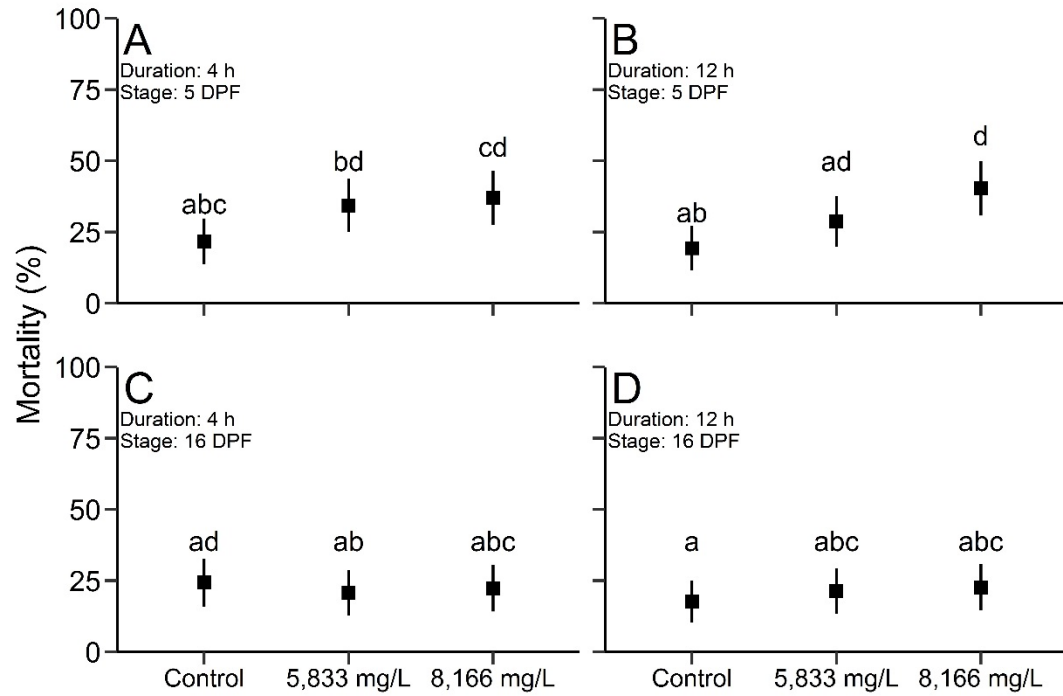


Figure 13. Percent mortality of Rainbow Trout embryos (mean $\pm$ 95% CL) exposed to gelatin for 4 h at 5 DPF (A), 12 h at 5 DPF (B), 4 h at 16 DPF (C), and 12 h at 16 DPF (D). Means denoted by the same letters were not statistically different.

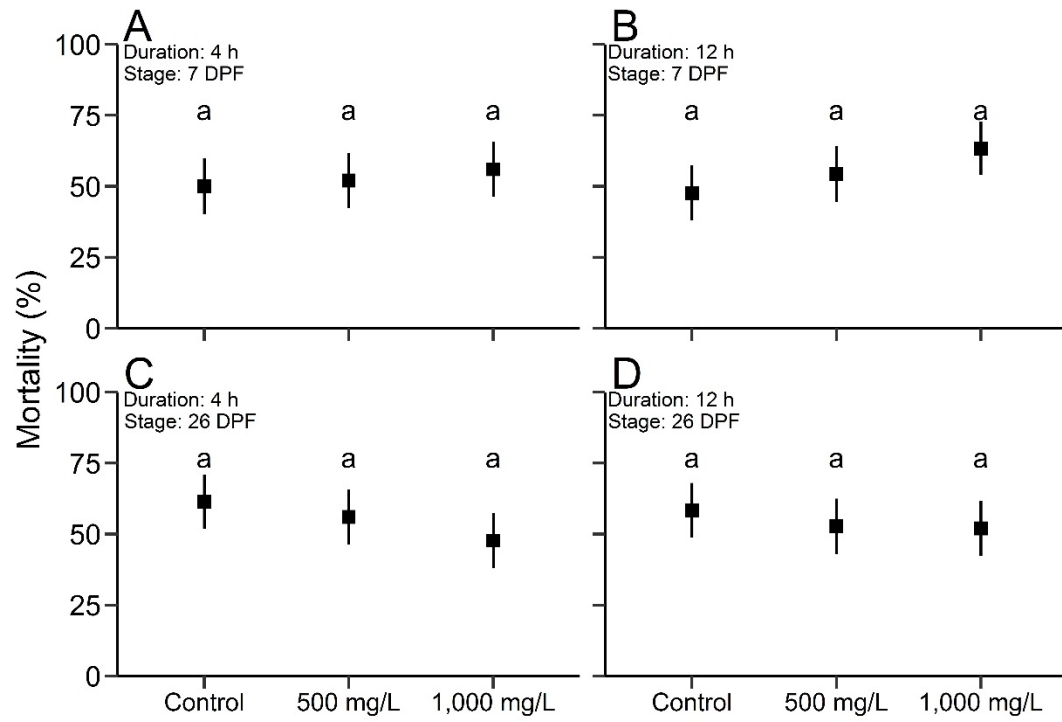


Figure 14. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) exposed to gelatin for 4 h at 7 DPF (A), 12 h at 7 DPF (B), 4 h at 26 DPF (C), and 12 h at 26 DPF (D). Means denoted by the same letters were not statistically different.

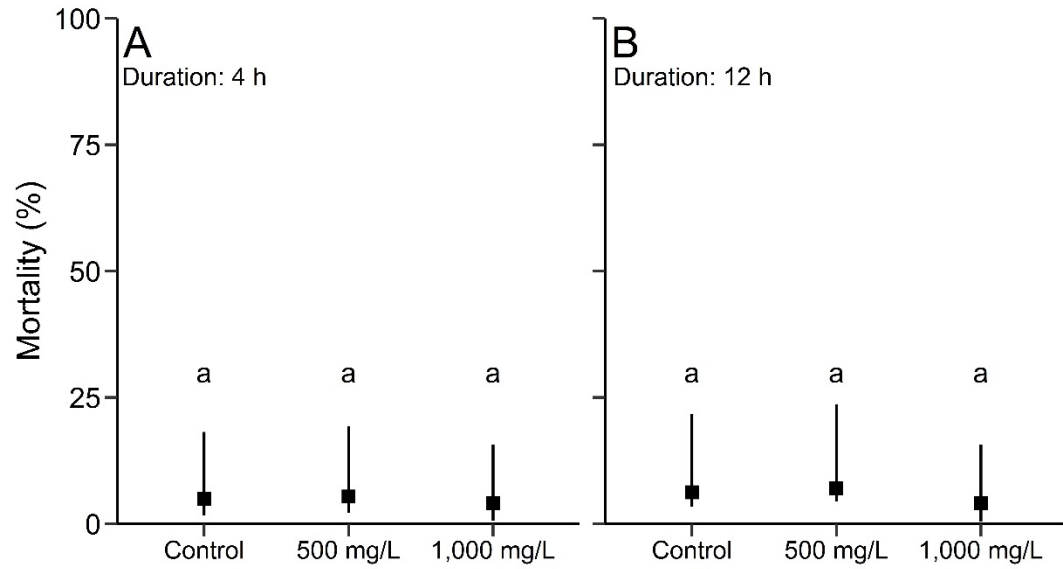


Figure 15. Percent mortality of Lake Trout larvae (mean $\pm$ 95% CL) exposed to gelatin for 4 h (A), and 12 h (B). Means denoted by the same letters were not statistically different.

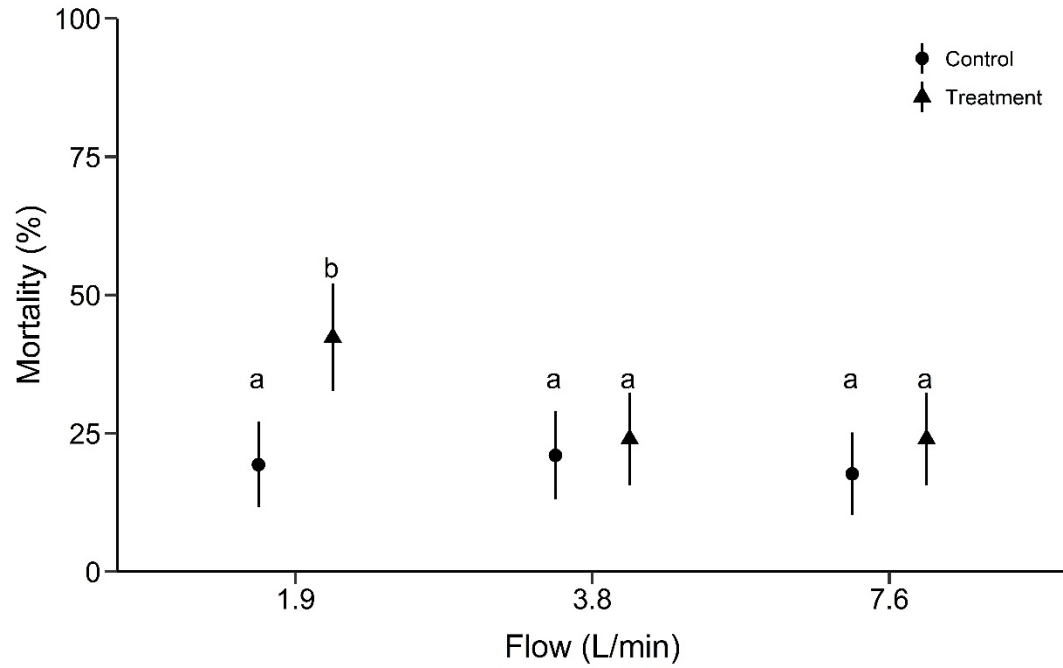


Figure 16. Percent mortality of Rainbow Trout embryos (mean $\pm$ 95% CL) exposed to one whole Rainbow Trout carcass at three flow rates. Control embryos are depicted by circles and treatment embryos by triangles. Means denoted by the same letters were not statistically different.

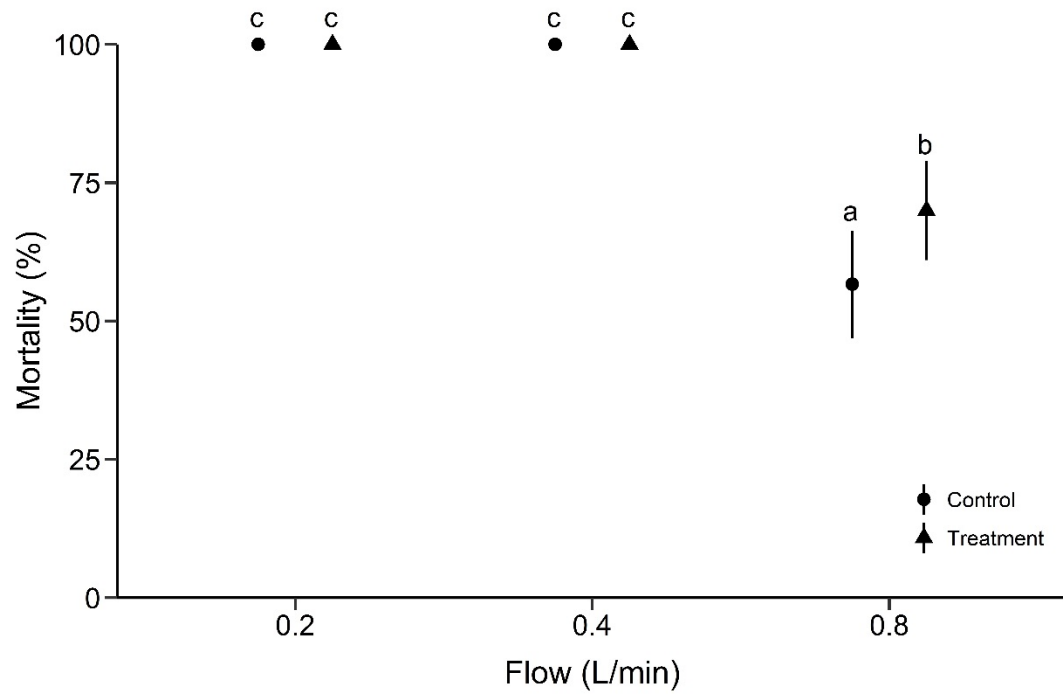


Figure 17. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) exposed to one whole Rainbow Trout carcass at three flow rates. Control embryos are depicted by circles and treatment embryos by triangles. Means denoted by the same letters were not statistically different

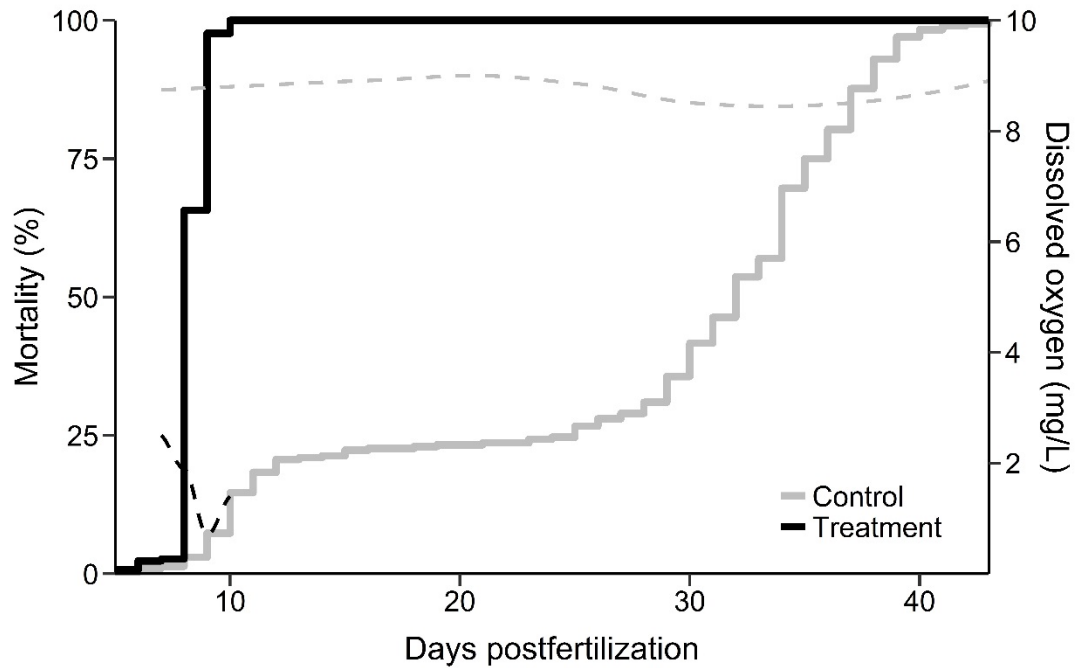


Figure 18. Cumulative mean percent mortality of Lake Trout embryos exposed to one whole Rainbow Trout carcass at 0.2 L/min. Mean control embryo mortality is depicted by the solid gray line and mean treatment mortality by the solid black line. Secondary y-axis depicts daily dissolved oxygen concentrations of trays containing control (gray dashed line) and treatment (black dashed line) embryos.

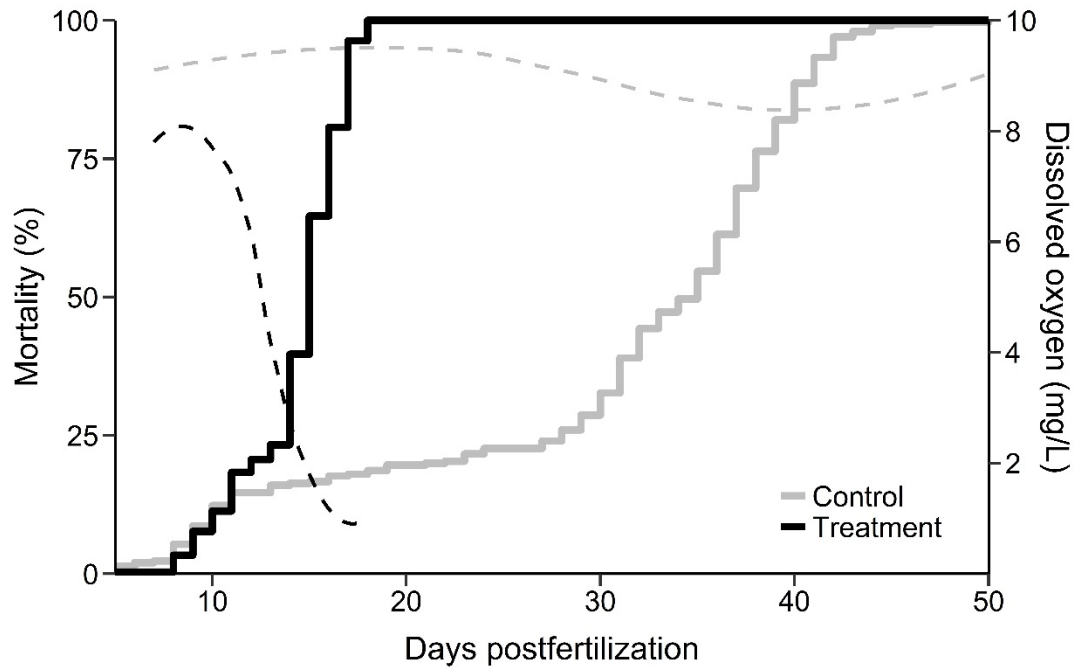


Figure 19. Cumulative mean percent mortality of Lake Trout embryos exposed to one whole Rainbow Trout carcass at 0.4 L/min. Mean control embryo mortality is depicted by the solid gray line and mean treatment mortality by the solid black line. Secondary y-axis depicts daily dissolved oxygen concentrations of trays containing control (gray dashed line) and treatment (black dashed line) embryos.

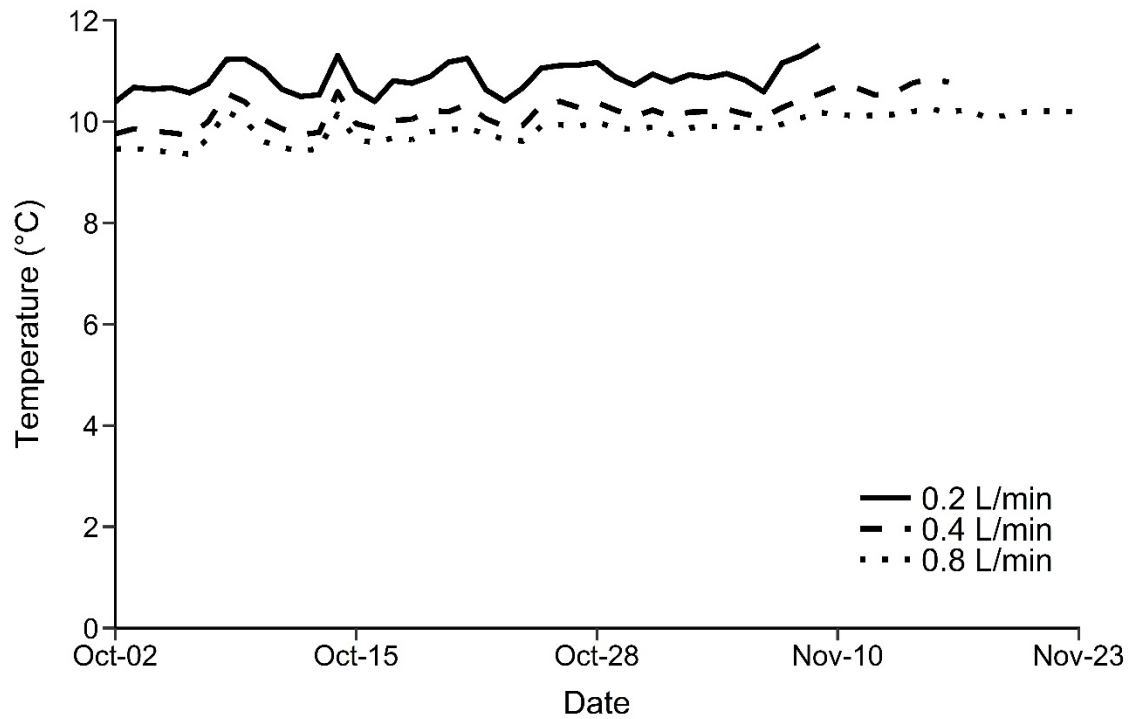


Figure 20. Daily temperatures (°C) in whole carcass Lake Trout embryo experiments. Solid line depicts the 0.2 L/min treatment, dashed line depicts the 0.4 L/min treatment, and dotted line depicts the 0.8 L/min treatment.

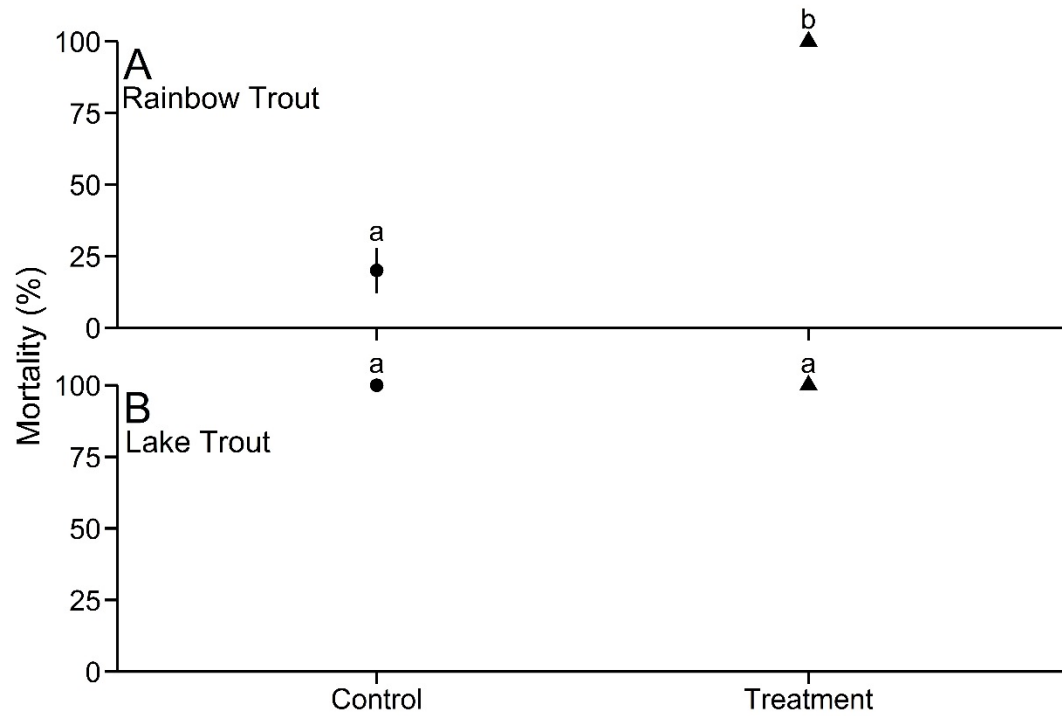


Figure 21. Percent mortality of Rainbow Trout embryos (mean $\pm$ 95% CL; A) and Lake Trout embryos (B) exposed to 10 g Rainbow Trout carcass musculature in static exposures. Control embryos are depicted by circles and treatment embryos by triangles. Means denoted by the same letters were not statistically different.

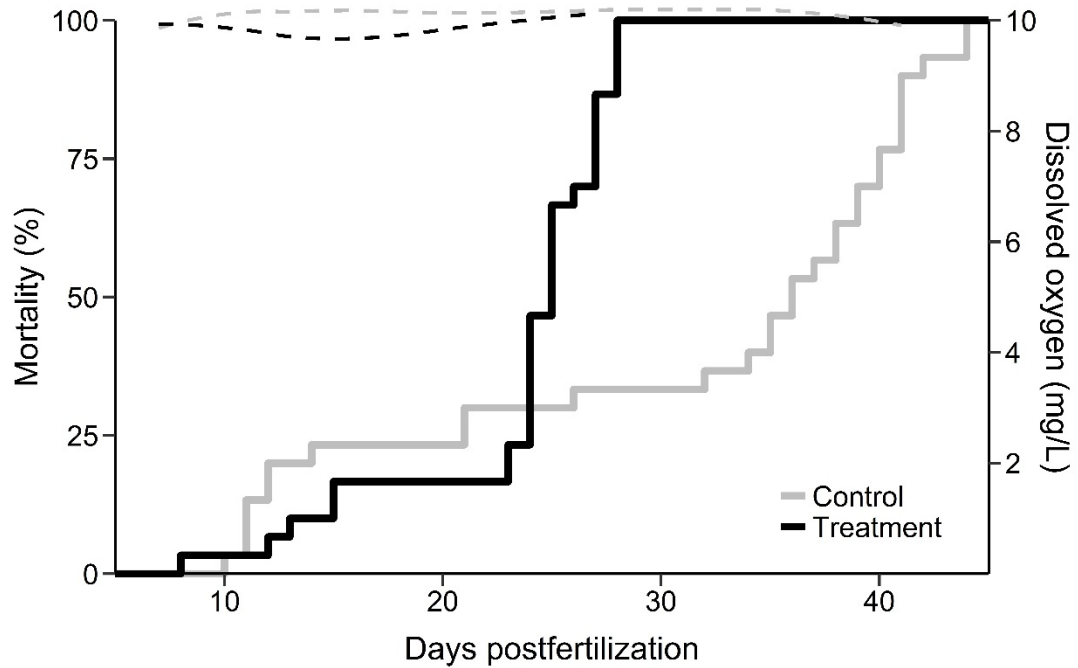


Figure 22. Cumulative mean percent mortality of Lake Trout embryos exposed to 10 g Rainbow Trout carcass musculature in static exposures. Mean control embryo mortality is depicted by the solid gray line and mean treatment mortality by the solid black line. Secondary y-axis depicts daily dissolved oxygen concentrations of exposure bins containing control (gray dashed line) and treatment (black dashed line) embryos.

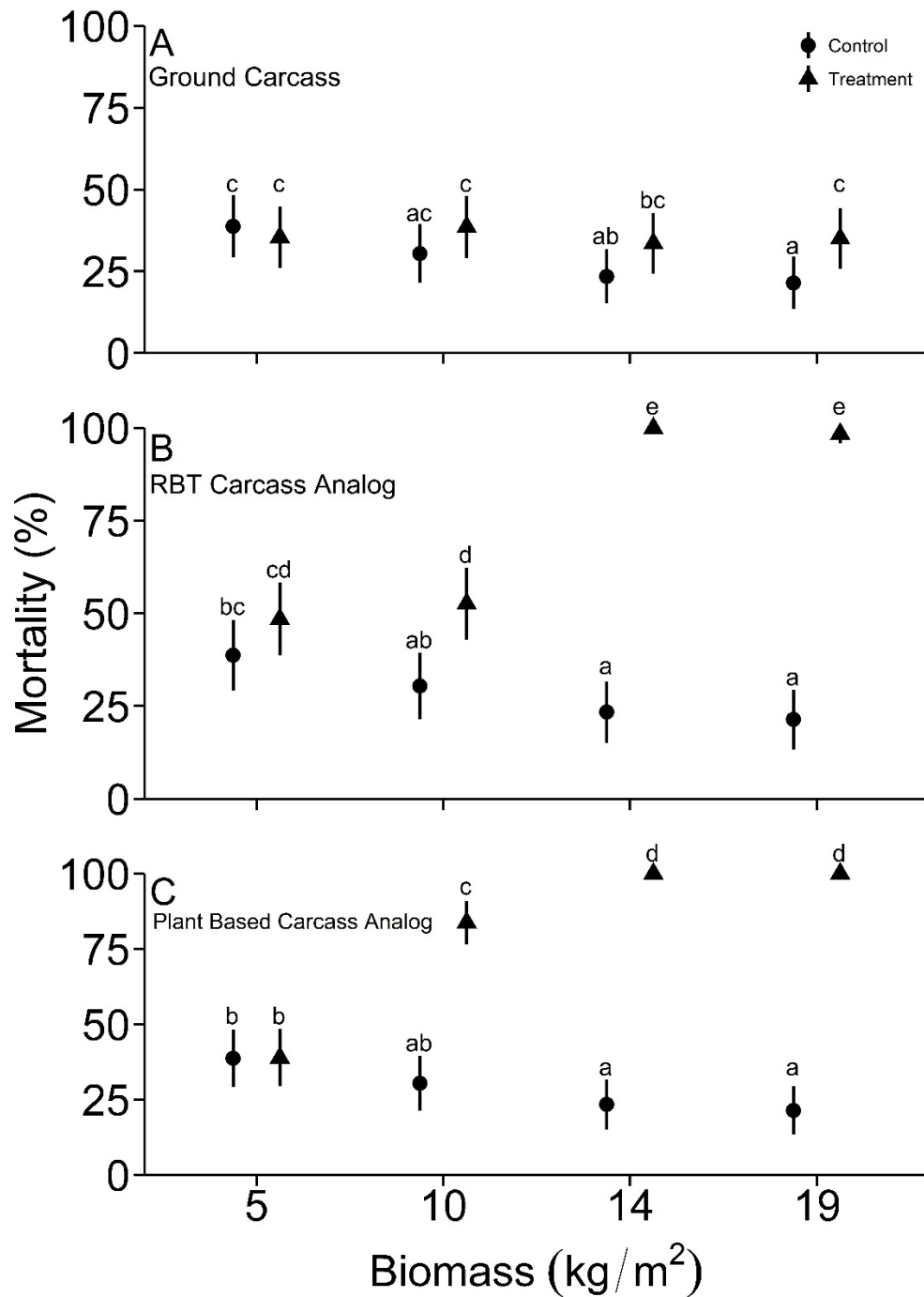


Figure 23. Percent mortality of Rainbow Trout (RBT) embryos (mean $\pm$ 95% CL) exposed to ground Rainbow Trout carcass material (A), Rainbow Trout carcass analogs (B), and plant-based carcass analogs (C) at four biomass levels. Control embryos are depicted by circles and treatment embryos by triangles. Means denoted by the same letters were not statistically different.

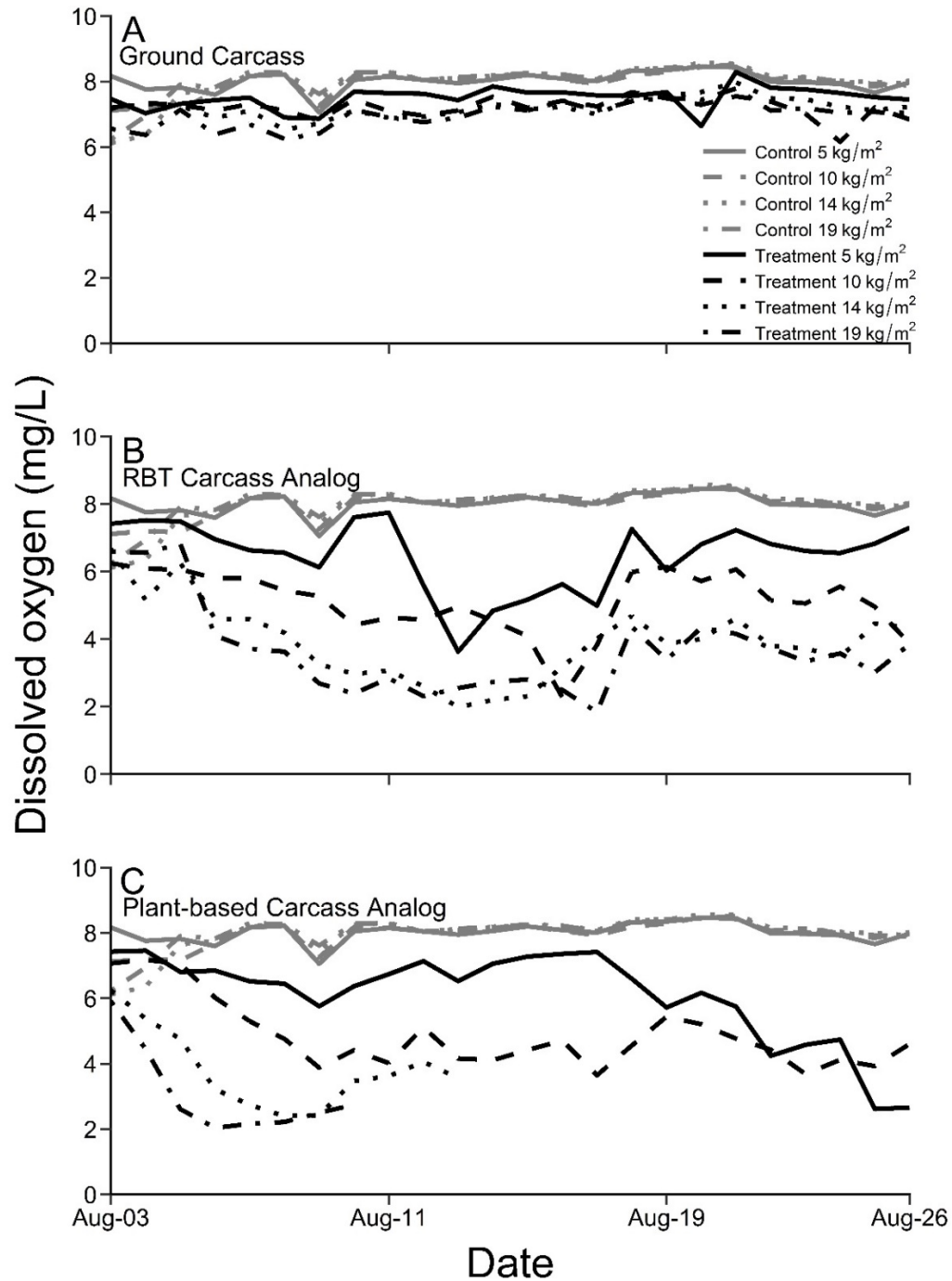


Figure 24. Dissolved oxygen concentrations in ground carcass (A), Rainbow Trout (RBT) carcass analog (B), and plant-based carcass analog (C) exposures at four biomass levels. Solid lines depict 5 kg/m², dashed lines depict 10 kg/m², dotted lines depict 14 kg/m², and dot-dash lines depict 19 kg/m². Gray lines represent control dissolved oxygen concentrations and black lines represent treatment dissolved oxygen concentrations.

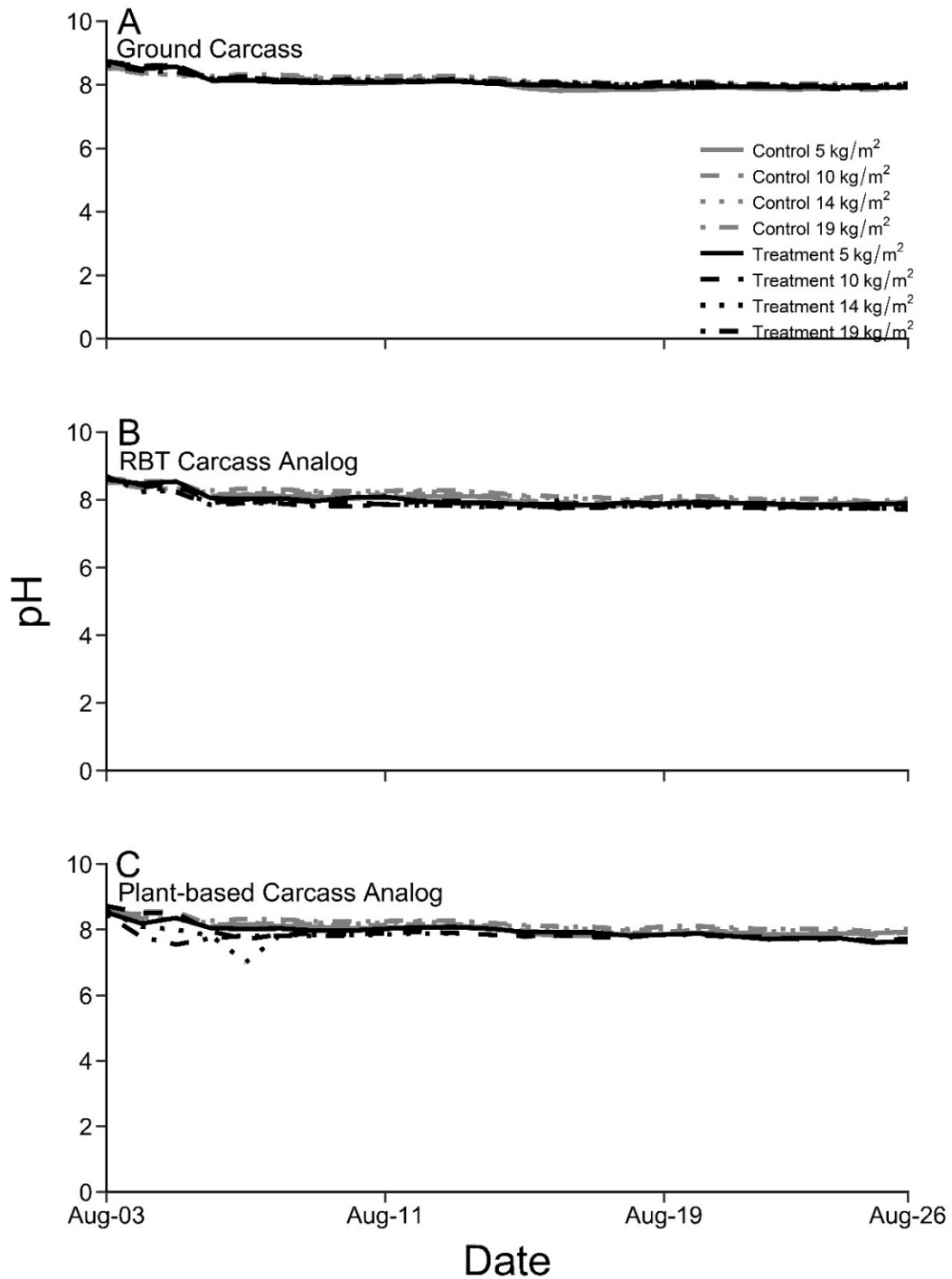


Figure 25. Daily pH in ground carcass (A), Rainbow Trout (RBT) carcass analog (B), and plant-based carcass analog (C) exposures at four biomass levels. Solid lines depict 5 kg/m², dashed lines depict 10 kg/m², dotted lines depict 14 kg/m², and dot-dash lines depict 19 kg/m². Gray lines represent control pH and black lines represent treatment pH.

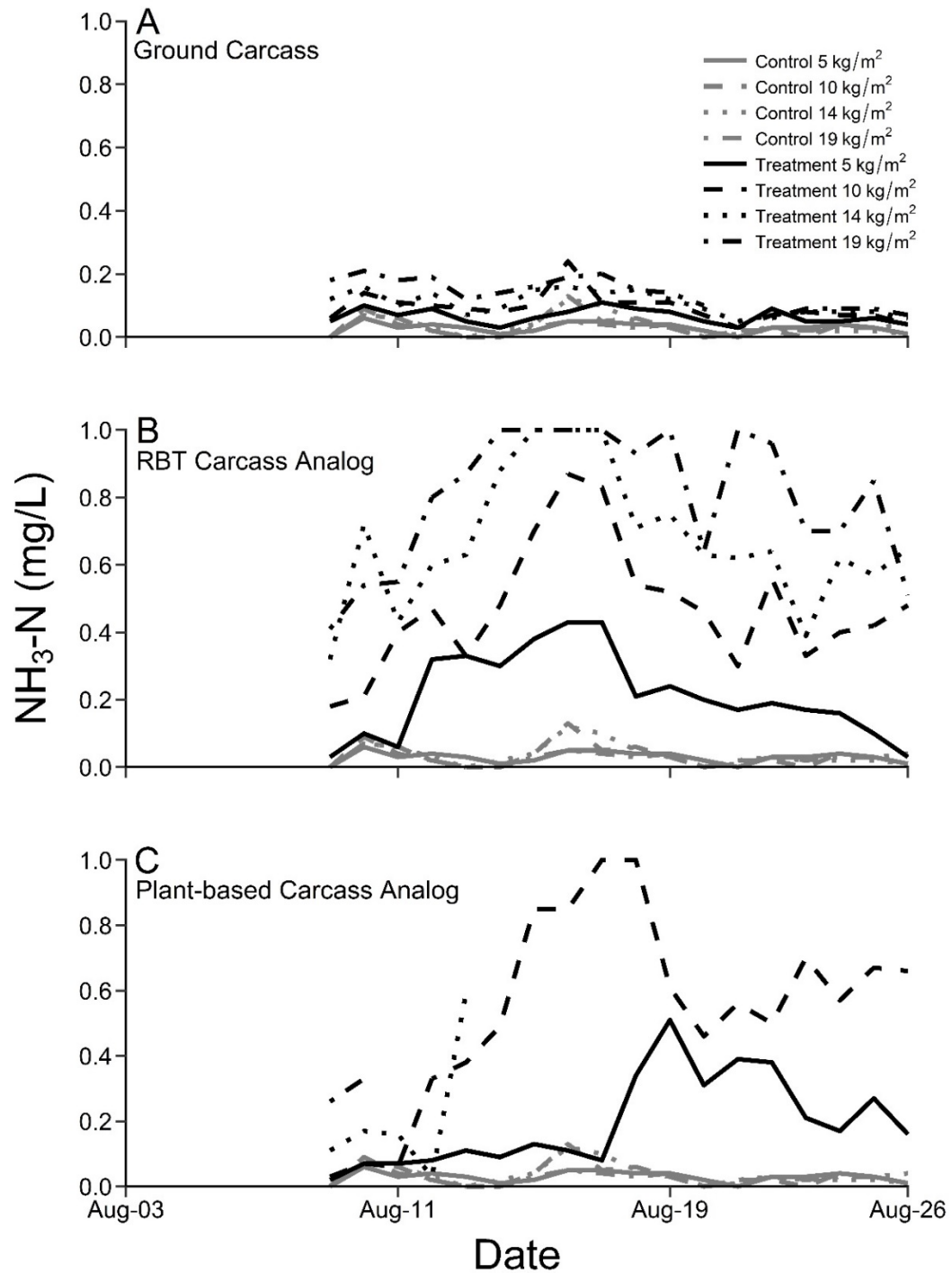


Figure 26. Ammonia concentrations in ground carcass (A), Rainbow Trout (RBT) carcass analog (B), and plant-based carcass analog (C) exposures at four biomass levels. Solid lines depict 5 kg/m², dashed lines depict 10 kg/m², dotted lines depict 14 kg/m², and dot-dash lines depict 19 kg/m². Gray lines represent control ammonia concentrations and black lines represent treatment ammonia concentrations.

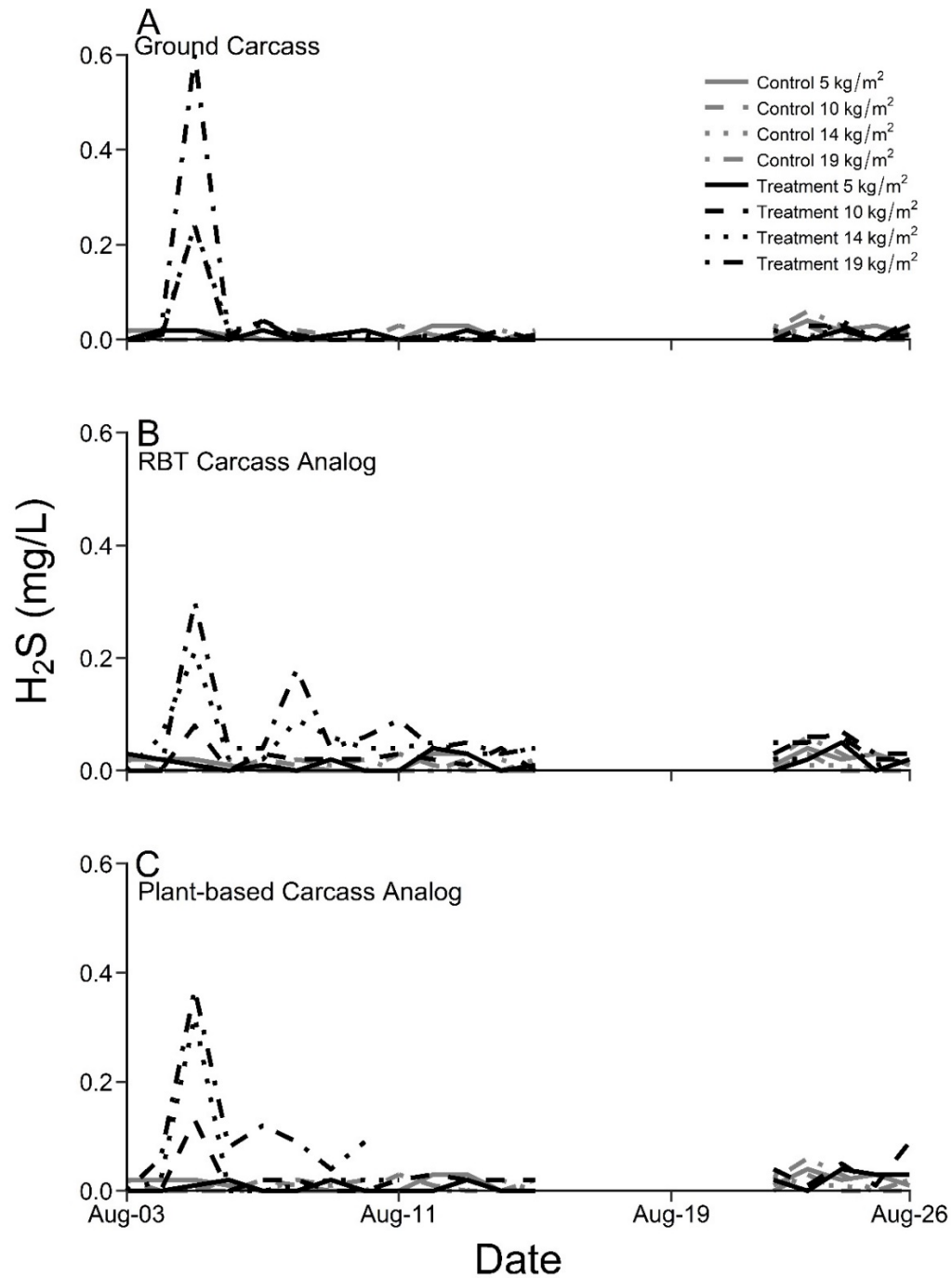


Figure 27. Hydrogen sulfide concentrations in ground carcass (A), Rainbow Trout (RBT) carcass analog (B), and plant-based carcass analog (C) exposures at four biomass levels. Solid lines depict 5 kg/m², dashed lines depict 10 kg/m², dotted lines depict 14 kg/m², and dot-dash lines depict 19 kg/m². Gray lines represent control hydrogen sulfide concentrations and black lines represent treatment hydrogen sulfide concentrations.

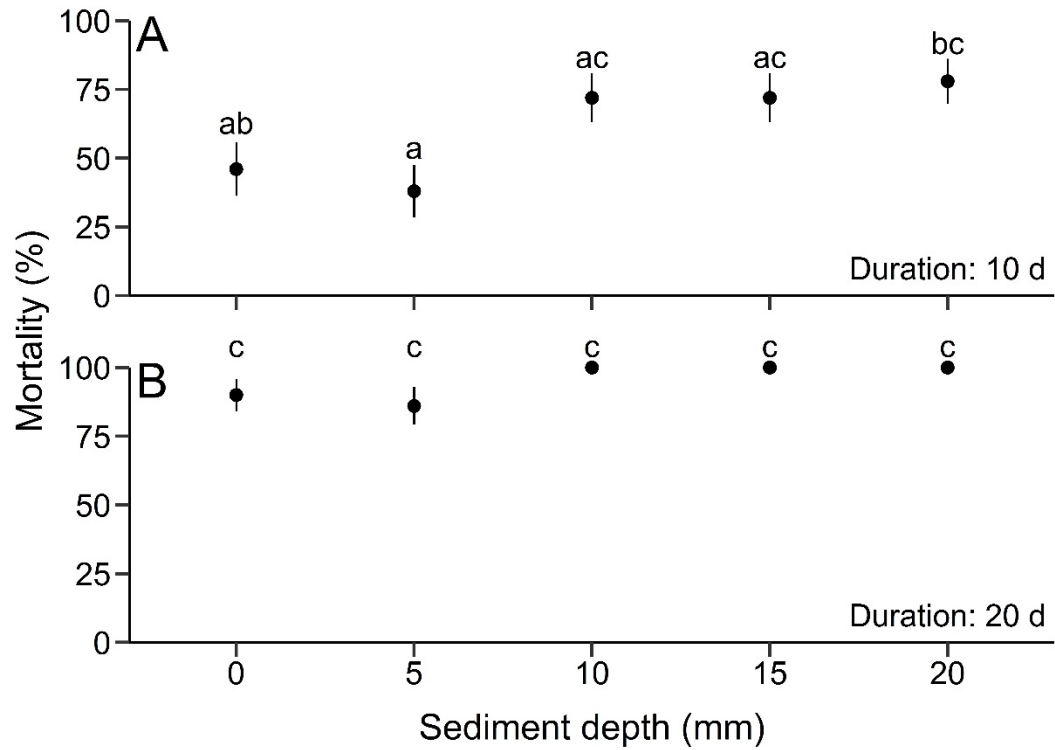


Figure 28. Percent mortality of Rainbow Trout embryos (mean $\pm$ 95% CL) exposed to five levels of sediment depth for 10 (A) and 20 days (B). Means denoted by the same letters were not statistically different.

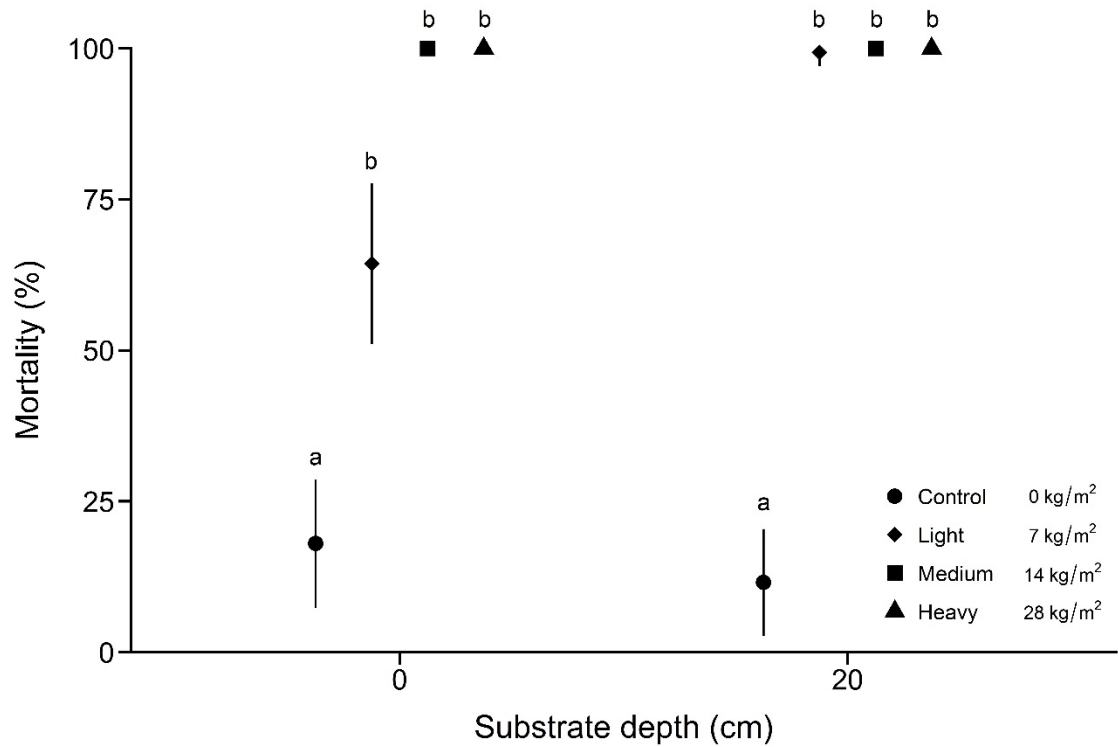


Figure 29. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) in short-term incubators at 0 cm (substrate surface) and 20 cm in the substrate in control sites (circles), light treatment sites (diamonds) treated with 7 kg/m² of ground carcasses, medium treatment sites (squares) treated with 14 kg/m² of ground carcasses, or heavy treatment sites (triangles) treated with 28 kg/m² of ground carcasses. Short-term experiments were conducted in autumn 2017 for 19 days at Thomas Bank, Yellowstone Lake, Yellowstone National Park. Means denoted by the same letters were not statistically different.

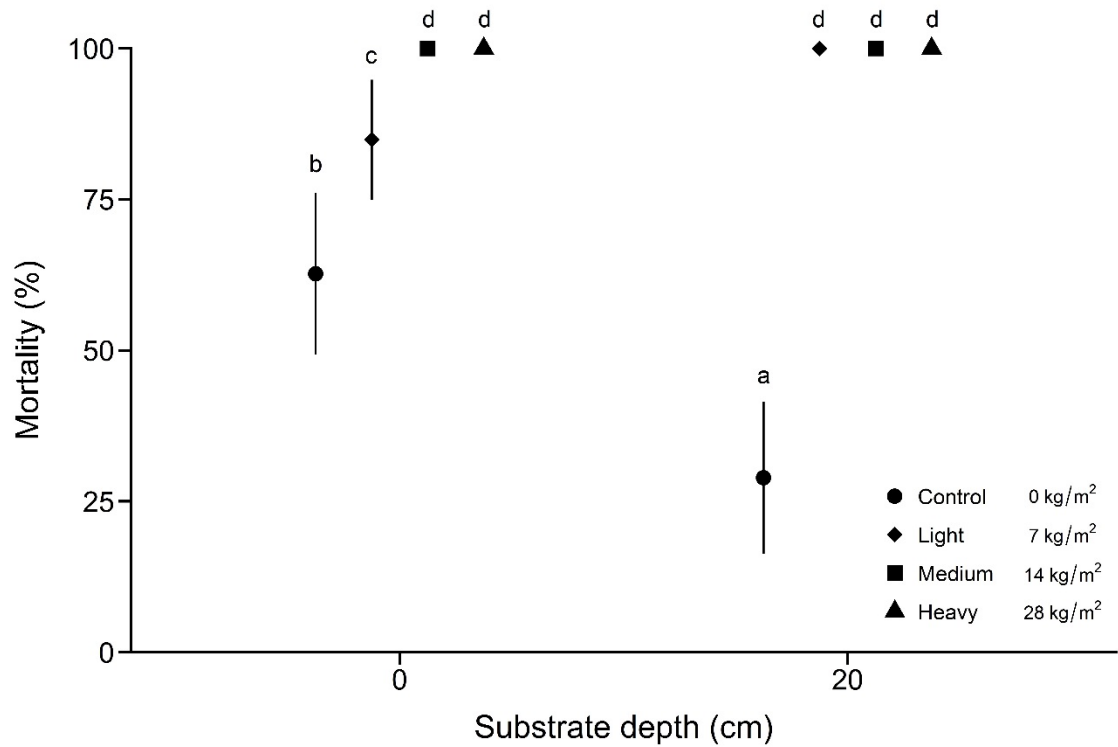


Figure 30. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) in long-term incubators at 0 cm (substrate surface) and 20 cm in the substrate in control sites (circles), light treatment sites (diamonds) treated with 7 kg/m² of ground carcasses, medium treatment sites (squares) treated with 14 kg/m² of ground carcasses, or heavy treatment sites (triangles) treated with 28 kg/m² of ground carcasses. Long-term experiments were conducted from September 2017 through June 2018 for 256 days at Thomas Bank, Yellowstone Lake, Yellowstone National Park. Means denoted by the same letters were not statistically different.

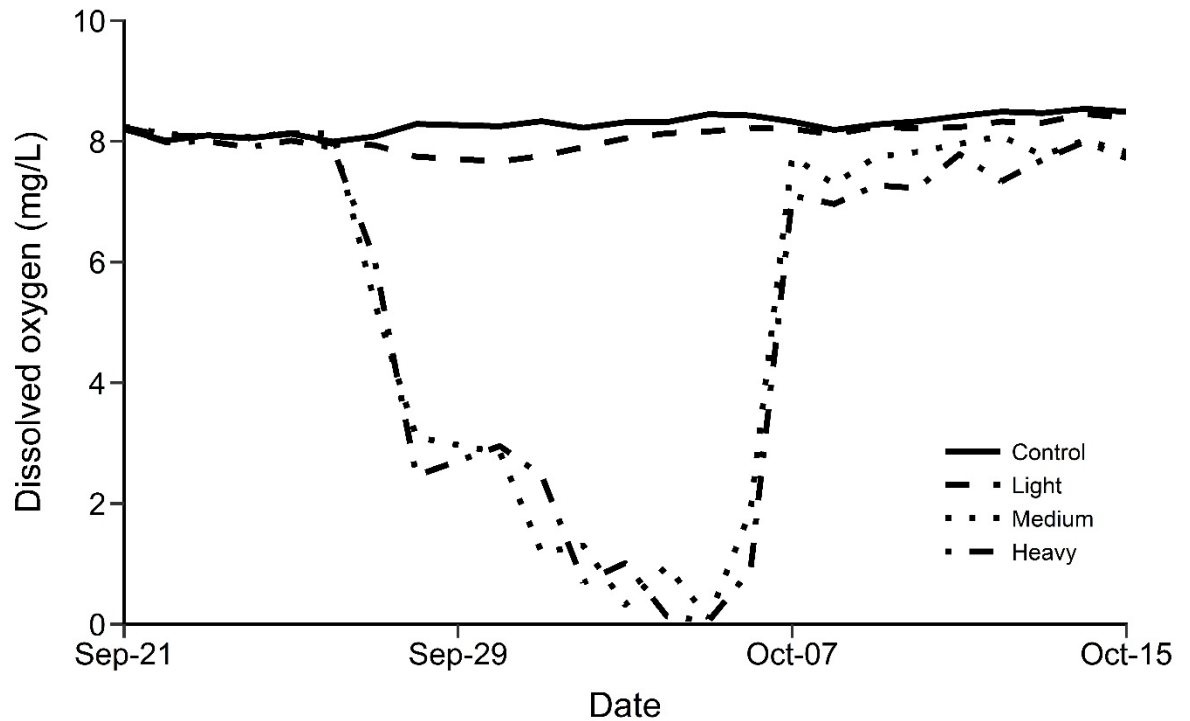


Figure 31. Dissolved oxygen concentrations (mg/L) during short-term experiments at the substrate surface within control sites (solid line), light treatment sites (dashed line) treated with 7 kg/m² of ground Lake Trout carcasses, medium treatment sites (dotted line) treated with 14 kg/m² of ground carcasses, and heavy treatment sites (dot-dash line) treated with 28 kg/m² of ground carcasses. Ground carcasses were placed on the substrate at treatment sites on September 27. Short-term incubators were recovered on October 16.

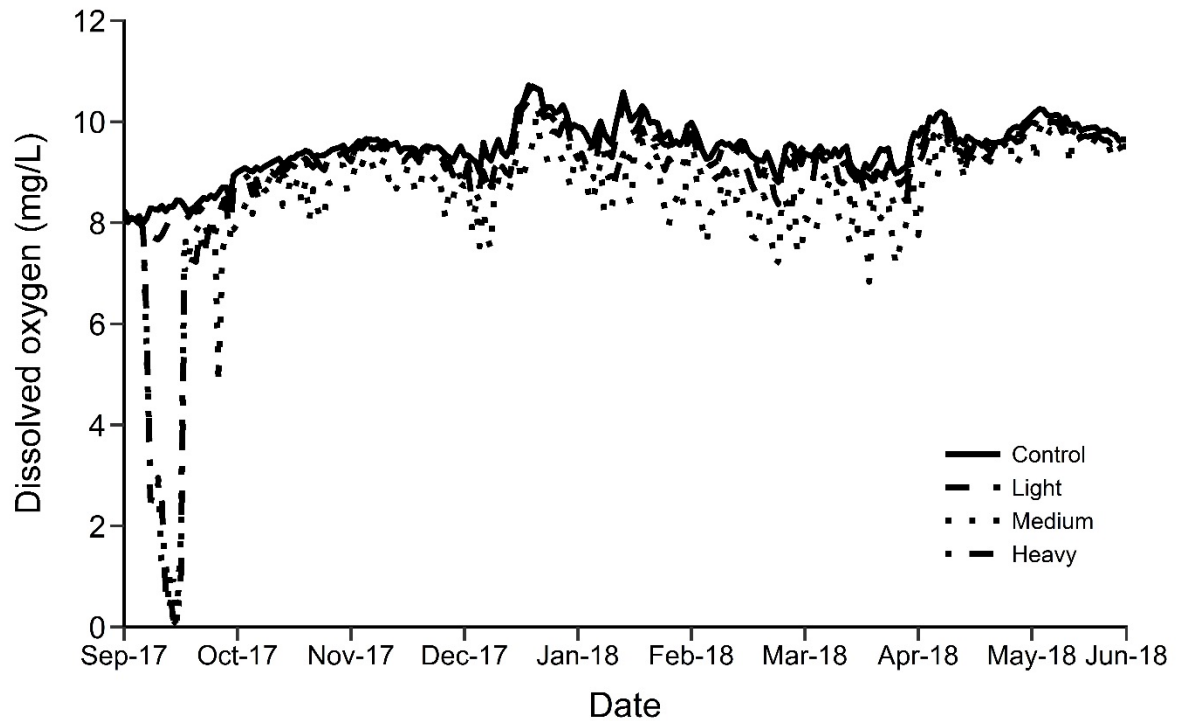


Figure 32. Dissolved oxygen concentrations (mg/L) during long-term experiments at the substrate surface within control sites (solid line), light treatment sites (dashed line) treated with 7 kg/m² of ground Lake Trout carcasses, medium treatment sites (dotted line) treated with 14 kg/m² of ground carcasses, and heavy treatment sites (dot-dash line) treated with 28 kg/m² of ground carcasses. Ground carcasses were placed on the substrate at treatment sites on September 27, 2017. Long-term incubators were recovered on June 14, 2018.

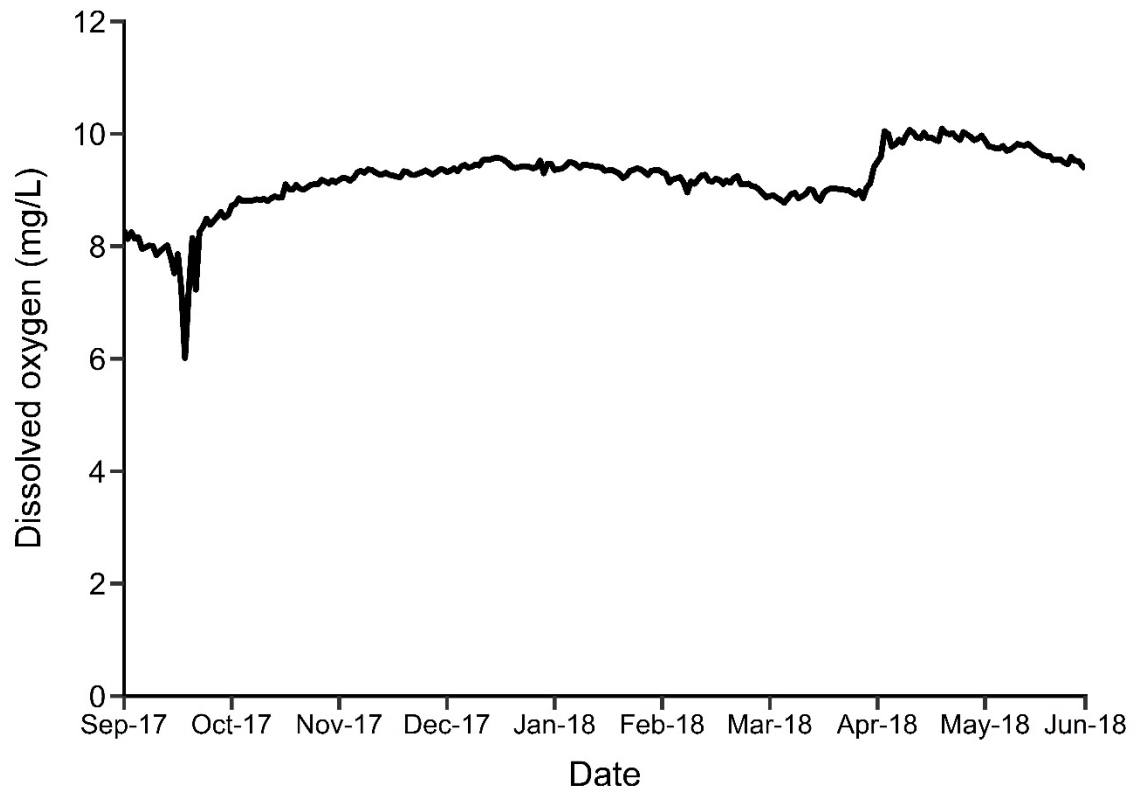


Figure 33. Dissolved oxygen concentrations during deep-water ground Lake Trout carcass deposition experiments at Olson Reef, Yellowstone Lake, Yellowstone National Park. About 2,156 kg of ground carcass material was deposited at Olson Reef over a 6-d period from October 2, 2017 to October 7, 2017.

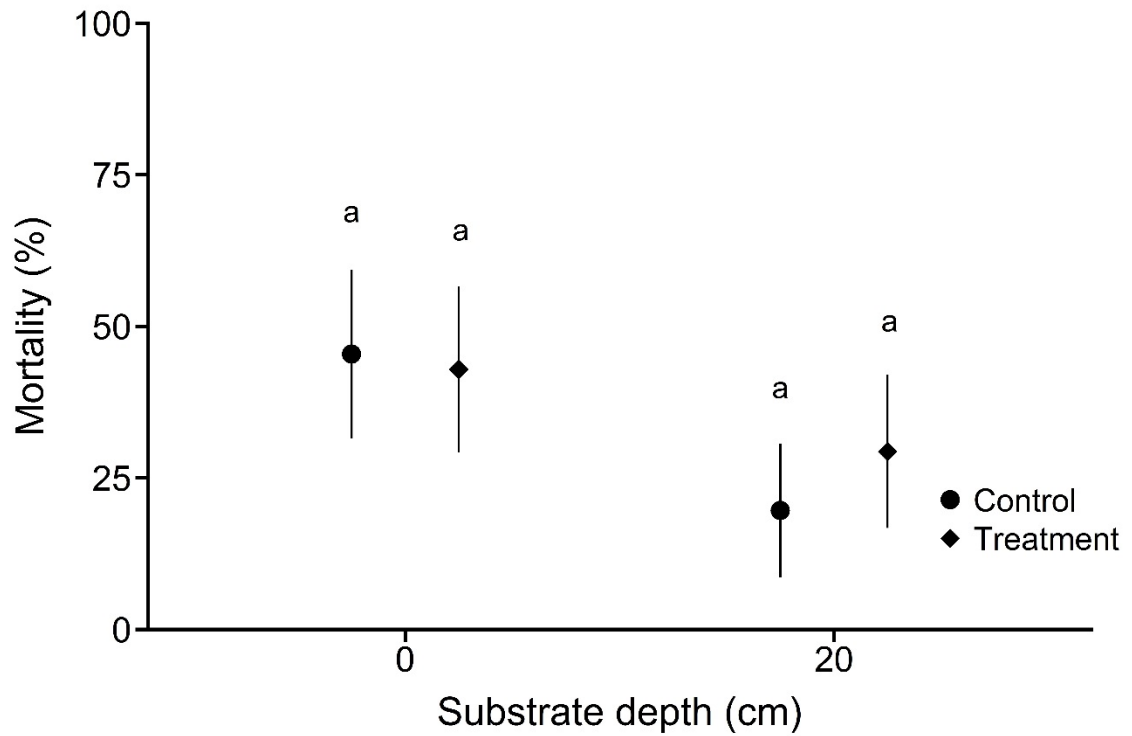


Figure 34. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) in short-term incubators at 0 cm (substrate surface) and 20 cm in the substrate in control sites (circles) and treatment sites (diamonds) in sediment deposition experiments. Short-term experiments were conducted in autumn 2017 for 18 days at Carrington Island, Yellowstone Lake, Yellowstone National Park. Means denoted by the same letters were not statistically different.

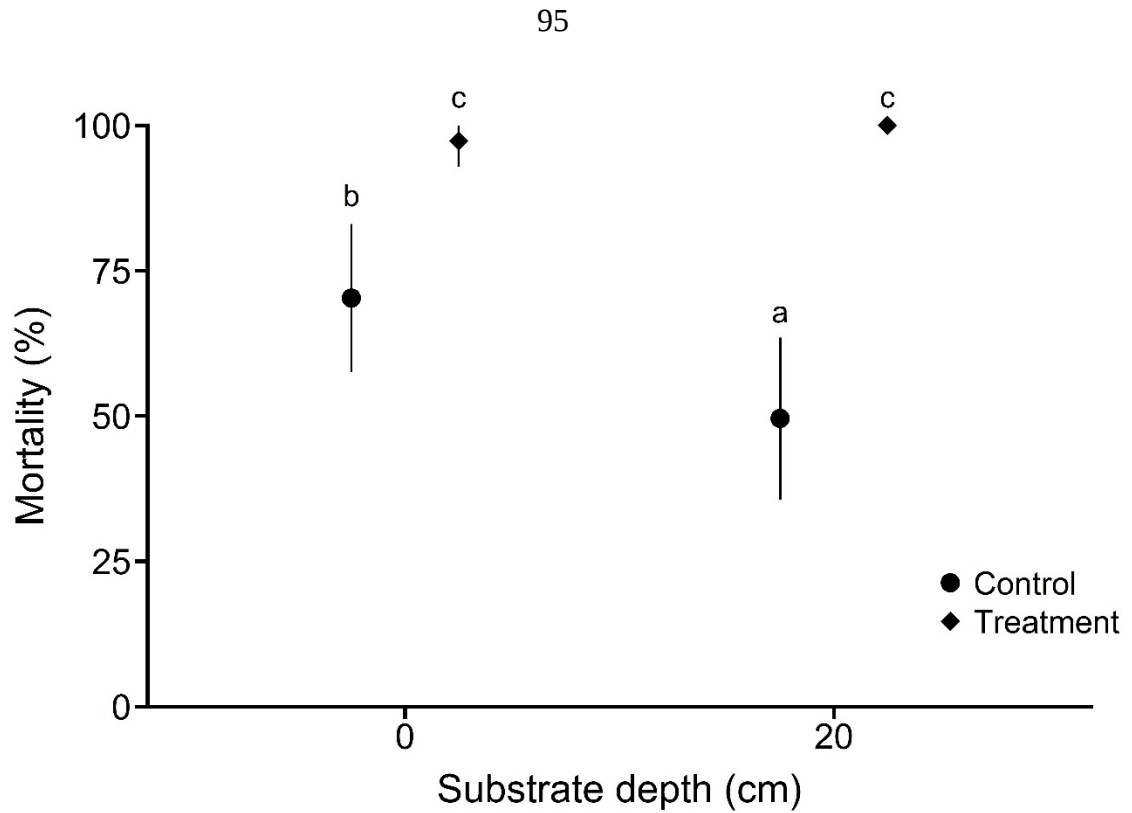


Figure 35. Percent mortality of Lake Trout embryos (mean $\pm$ 95% CL) in long-term incubators at 0 cm (substrate surface) and 20 cm in the substrate in control sites (circles) and treatment sites (diamonds) in sediment deposition experiments. Long-term experiments were conducted from September 2017 through June 2018 for 249 days at Carrington Island, Yellowstone Lake, Yellowstone National Park. Means denoted by the same letters were not statistically different.

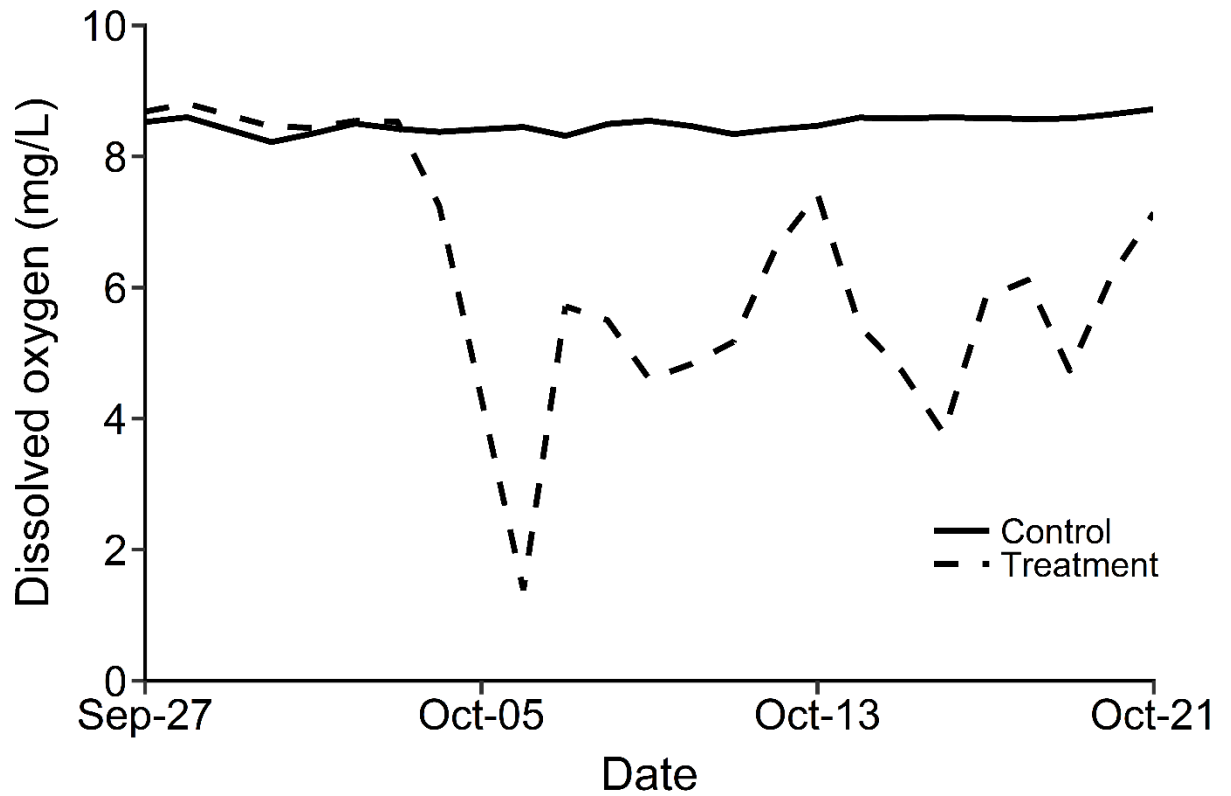


Figure 36. Dissolved oxygen concentrations (mg/L) during short-term experiments at the substrate surface within control sites (solid line) and treatment sites (dashed line) at sediment deposition experiment sites at Carrington Island, Yellowstone Lake. Sediment was deposited at treatment sites on October 5. Short-term incubators were recovered on October 23.

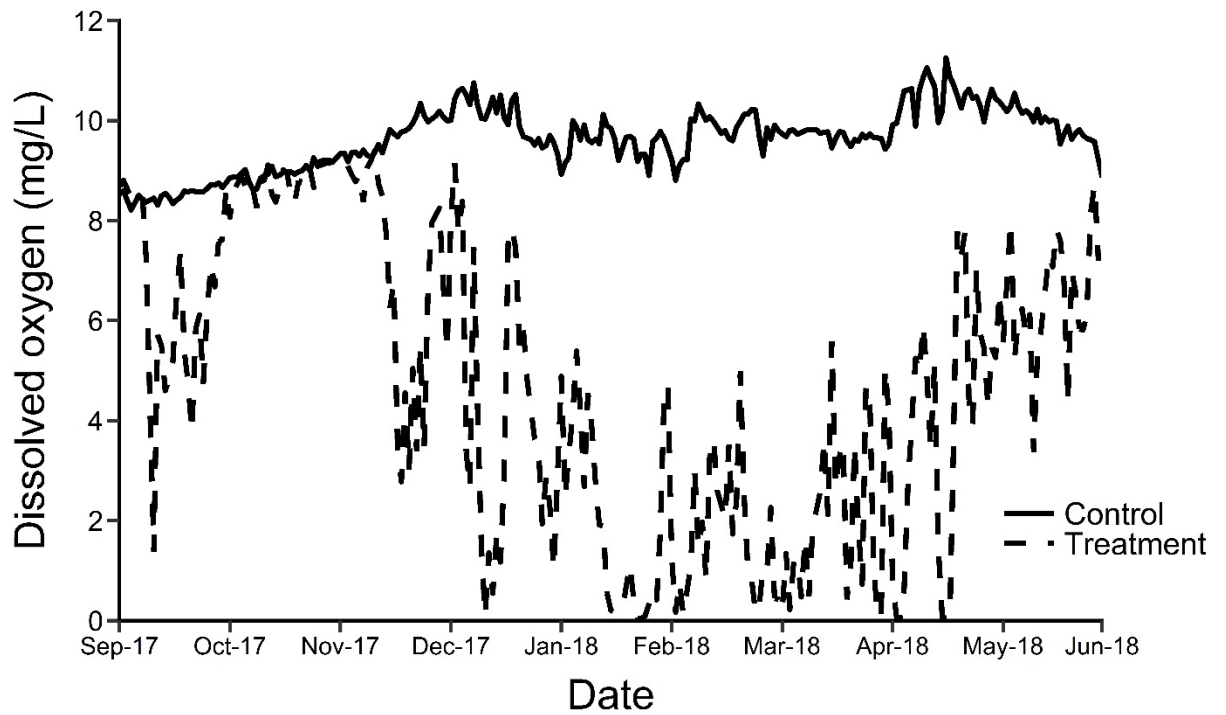


Figure 37. Dissolved oxygen concentrations (mg/L) during long-term experiments at the substrate surface within control sites (solid line) and treatment sites (dashed line) at sediment deposition experiment sites at Carrington Island, Yellowstone Lake. Sediment was deposited at treatment sites on October 5, 2017. Long-term incubators were recovered on June 12, 2018.

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