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Organic Pellet Decomposition Induces Mortality of Lake Trout Embryos in Yellowstone Lake

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Abstract

   Yellowstone Lake is the site of actions to suppress invasive Lake Trout *Salvelinus namaycush* and restore native Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* and natural ecosystem function. Although gill netting is effective (Lake Trout λ ≤ 0.6 from 2012 through 2018), the effort costs more than US\$2 million annually and only targets Lake Trout age 2 and older. To increase suppression efficiency, we developed an alternative method using organic (soy and wheat) pellets to increase mortality of Lake Trout embryos on spawning sites. Decomposition of pellets during two in situ experiments caused dissolved oxygen (DO) concentrations to temporarily decline to lethal levels (<3.4 mg/L) within days of application. Embryo mortalities during the first exposure period (days 1–6 following application) were high at all treatment levels (1.75–28.0 kg/m^2) at the substrate surface and within interstices 20 cm below the surface, varying from 97 ± 1.8% (mean ± SE) to 100 ± 0.0%, but may have been enhanced by a handling effect (exposure to sunlight). Embryo mortalities during the second exposure period (days 11–22) were highest 20 cm below the surface, varying from 78 ± 9.7% to 100 ± 0.0%. Almost all (98 ± 3.1%) Lake Trout embryos died after exposure to DO < 3.4 mg/L for >200 h during the second period. Pellets caused lethal DO for several weeks below the substrate surface, despite largely dissolving and dissipating from the surface of treated areas by day 39. Broad-scale application of pellets at 1.75 kg/m^2 following the spawning period in autumn may reduce Lake Trout recruitment and enhance population suppression because the area of 14 verified spawning sites is only 11.4 ha (0.03% of lake surface area). Pellet application may be useful in other similar systems as part of an integrated pest management approach targeting multiple life stages of invasive freshwater fish.

Introduced, nonnative fish have disrupted freshwater ecosystems throughout the world by eliminating native species and altering aquatic communities (Vander Zanden et al. 1999; Cucherousset and Olden 2011). Predatory fish introductions have been particularly detrimental in the western United States, where native fish species richness is naturally low (Clarkson et al. 2005). Because of their popularity as sport fish, nonnative predatory fish have been widely introduced, both intentionally by managers (McMahon and Bennett 1996) and illegally by anglers (Rahel 2004), and have also dispersed naturally via connected waterways (Muhlfeld et al. 2011; Rahel and Smith 2018). Along with causing displacement or extirpation or both of native fishes, introduced predatory fish can add a previously nonexistent trophic level that exerts cascading, top-down effects and alters aquatic food webs (Eby et al. 2006). Managers have taken aggressive actions to suppress introduced predatory fish and restore native species and natural ecological processes (Mueller 2005; Weidel et al. 2007).

Yellowstone Lake has been the site of intensive efforts to conserve native Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* (Behnke 2002; Gresswell 2011) and restore natural ecological function (Tronstad et al. 2010; Koel et al. 2019) since invasive Lake Trout *Salvelinus namaycush* were first discovered there in 1994 (Kaeding et al. 1996; Koel et al. 2005). Gillnetting was implemented in 1995 to suppress the Lake Trout population (Ruzycki et al. 2003), but despite annual increases in gillnetting effort (Syslo et al. 2011), the population expanded throughout Yellowstone Lake and increased in abundance until 2012, when the effort became large enough to curtail population growth (Koel et al. 2019). The Lake Trout population of Yellowstone Lake is highly resilient to gillnetting, probably because of high early life

history survival (Syslo et al. 2011; Cox et al. 2013). Interstitial embryo predators, which are a common source of embryo mortality in the native range of Lake Trout (Claramunt et al. 2005), do not inhabit Yellowstone Lake (Varley and Schullery 1998). Because Lake Trout population growth rates are most sensitive to changes in age-0 survival (Ferreri et al. 1995; Cox et al. 2013; Fredenberg et al. 2017), alternative methods are being developed to reduce prerecruit survival (Brown et al. 2017; Doepke et al. 2017; Thomas et al. 2019), with an overall goal of increasing suppression efficiency while reducing long-term costs.

We sought to intentionally degrade interstitial water quality at Lake Trout spawning sites in Yellowstone Lake because salmonid embryos are highly susceptible to sedimentation and degraded water quality (Gunn and Keller 1984; Haines and Baker 1986; Sly 1988). Rapid mortality of Lake Trout embryos occurs when dissolved oxygen (DO) concentrations are below 3.4 mg/L (Garside 1959; Carlson and Siefert 1974). Whole and ground Lake Trout carcasses caused 100% mortality of Lake Trout embryos at the substrate surface and at a depth of 20 cm in the substrate at treated spawning sites (Poole 2019; Thomas et al. 2019). Biological oxygen demand of the decomposing carcasses caused DO to decline to 0 mg/L soon after treatments and caused the high embryo mortality.

Although these previous studies suggested that Lake Trout carcasses may be a useful embryo suppression tool, the Lake Trout spawning period peaks during the last week of September, allowing only 2–3 weeks to treat spawning sites prior to the end of gillnetting (which limits carcass availability) and onset of dangerous winter conditions on Yellowstone Lake. Lake Trout carcasses are also difficult to transport in large quantities in boats, time-consuming to apply, and prone to drift or dispersal by wildlife

from spawning sites. We therefore sought an analogous organic material that would alleviate these constraints. Pelletized carcass-analogs have been used to restore nutrients and ecosystem function in areas of the Pacific Northwest, where anadromous salmonids historically contributed to stream productivity through carcass decomposition (Wipfli et al. 2004; Pearsons et al. 2007). Fish-based and plant-based organic pellet formulations induced high mortality of embryos in laboratory bioassays by decreasing DO, increasing ammonia, and increasing hydrogen sulfide concentrations (Poole 2019). These experiments provided proof of concept for further in situ experiments in Yellowstone Lake.

Our goal was to develop an alternative Lake Trout suppression method that could be broadly implemented over a short period (2–3 weeks) using organic materials that are available during autumn currently and will be available in future years after Lake Trout population abundance has been effectively reduced by gillnetting (Koel et al. 2017). We selected plant-based organic (soy and wheat) pellets for in situ experiments on a Lake Trout spawning site in Yellowstone Lake (Thomas Bank; Figure 1) because they pose no risk of aquatic disease transfer. Our primary objectives were to determine the potential of organic
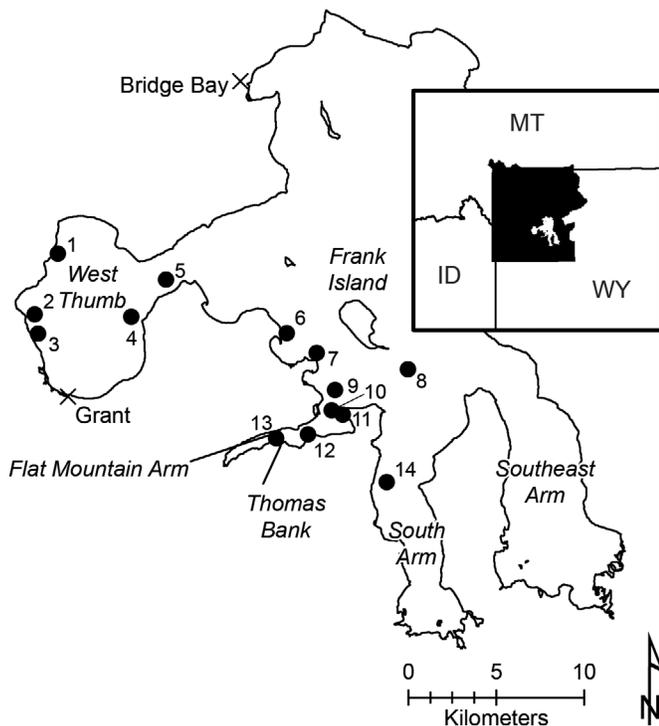

FIGURE 1. Map of Yellowstone Lake within Yellowstone National Park (the black area in the inset panel) and 14 known Lake Trout spawning sites (numbered black dots), including Thomas Bank in the Flat Mountain Arm, the site of in situ exposures of Lake Trout embryos to organic pellets. Spawning site numbers (1–14) refer to Table A.1 in the Appendix.

pellets to (1) reduce DO below lethal levels and (2) induce high mortality of Lake Trout embryos. We also sought to estimate the minimum organic pellet biomass and minimum exposure duration to lethal DO required to ensure near complete mortality of Lake Trout embryos in Yellowstone Lake.

STUDY AREA

Yellowstone Lake is at an elevation of 2,356 m in the east-central portion of Yellowstone National Park in northwestern Wyoming (Figure 1). Yellowstone Lake is the largest alpine (above 2,000 m) lake in North America and has a surface area of 341 km^2, mean depth of 48 m, and a maximum depth of 133 m (Kaplinski 1991). Most of the Yellowstone Lake watershed is within remote, protected federal wilderness. Ice covers the lake from late December through May, and the lake thermally stratifies from late July through early September, with a thermocline at a depth of 12–15 m. Surface water temperatures rarely exceed 18°C (in August; Koel et al. 2019). Specific conductance is typically <100 µS/cm (Koel et al. 2015).

Fourteen Lake Trout spawning sites were identified in Yellowstone Lake (Figure 1) over the past two decades by gillnetting spawning fish and locating telemetered fish. Spawning at these sites was verified by locating Lake Trout embryos by snorkeling (shallow sites), scuba diving, remotely operated vehicle imaging, or benthic sled sampling (Simard 2017). Depths of Lake Trout spawning sites vary from <1 to 30 m, and surface areas of sites where the outer perimeter has been delineated ($n = 11$) vary from 0.3 to 2.0 ha (total of 11.4 ha; Table A.1). Although some Lake Trout spawning site substrates consist of angular rock, most are embedded cobble, hardened material deposited by geothermal vents, bedrock, or some combination thereof (Table A.1) with little or no interstitial space, resulting in embryos remaining <20 cm deep in the substrate during development and hatching. Additional, as yet unverified, Lake Trout spawning sites probably exist (Williams 2019).

METHODS

Embryo exposures to organic pellets.— Embryos were produced from sexually mature Lake Trout obtained from suppression gill nets on Yellowstone Lake during September 2018. Fish were placed in holding tanks with lake water until sufficient numbers of males and females were obtained (about four 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. Embryos (i.e., fertilized prehatch embryos within egg envelopes;

Balon 1980) were incubated at 9°C in Living Stream recirculating tanks (Frigid Units, Inc.).

Plant-based, sinking pellets (1,500 kg) about 4.2-mm in diameter were produced at the Feed and Nutrition Laboratory of the Bozeman Fish Technology Center, U.S. Fish and Wildlife Service, in Montana (see Supplementary Material available in the online version of this article). In situ embryo exposures to pellets were conducted at a confirmed Lake Trout spawning site, the 0.71-km² Thomas Bank site in the Flat Mountain Arm (Figure 2; Table A.1), where a large talus slope of 5–50-cm-diameter angular rock extends from the mountainside into the lake. Thomas Bank is considered one of the most productive Lake Trout spawning sites in Yellowstone Lake because of its high-quality habitat. Abundant interstitial spaces among the clean, wave-swept rocks provide security for naturally broadcast Lake Trout eggs. Experimental plots, each measuring 3 × 3 m, were delineated with yellow polypropylene rope anchored to the substrate surface (Thomas et al. 2019). Three control plots and 15 pellet treatment plots (5 treatment levels, 3 replicates each) were distributed within a sampling frame that extended along 120 m of shoreline, about 10 m from shore, at water depths varying from 1.5 to 3.0 m. Plots of each treatment level (i.e., different pellet biomass densities) were grouped together and spatially distributed along the shoreline from the west end of the sampling frame (heavy treatment) in descending treatment levels to the east end (controls;
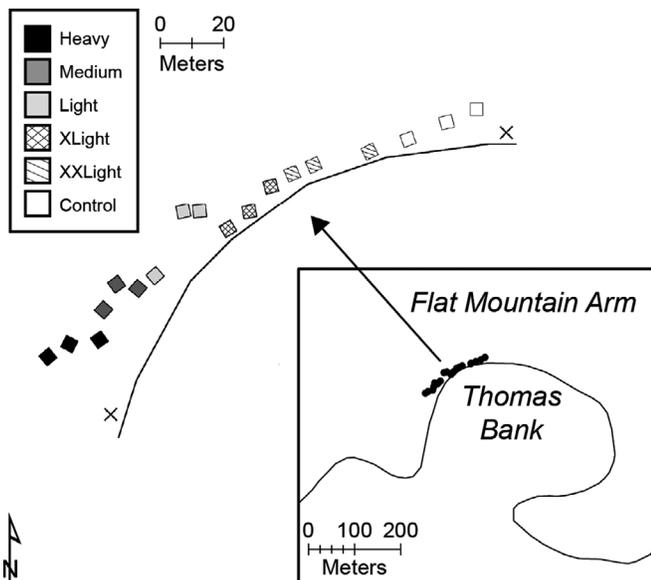

FIGURE 2. Map of the Lake Trout spawning site at Thomas Bank in the Flat Mountain Arm of Yellowstone Lake, showing the locations of the 9-m² plots ($n = 3$/treatment) used for in situ embryo exposures. The plots were treated with organic pellets at densities of 28.0 kg/m² (heavy), 14.0 kg/m² (medium), 7.0 kg/m² (light), 3.5 kg/m² (xlight), 1.75 kg/m² (xxlight), and control (no pellets).

Figure 2) to reduce effects of potential pellet drift among plots. No drift among plots was observed by scuba divers during the study period.

Plastic grid incubators (similar to Plexiglas incubators; Kennedy 1980; Gunn and Keller 1984) were used to hold Lake Trout embryos in the substrate within the experimental plots. Each incubator consisted of a 12- × 20- × 1.5-cm plastic grid panel containing 50 separate cells, each holding a single embryo. Cells were enclosed on both sides by a 12- × 20-cm fiberglass window-screen mesh (1-mm bar mesh) secured in place by two thinner plastic grid panels (12 × 20 × 0.75 cm) positioned on both sides of the middle panel and secured by stainless steel bolts and locking washers, making them negatively buoyant. Fifty live Lake Trout embryos were placed into each incubator and held in Living Stream tanks 24 h before they were placed in the experimental plots at Thomas Bank. Incubators were transported by boat to the experimental plots at Thomas Bank in containers filled with 9°C water, which was similar to the concurrent ambient water temperature in Yellowstone Lake.

Embryos were exposed to pellet treatments during two separate exposure periods: September 12–18 and September 23 to October 4, 2018. Embryos were 1 d postfertilization (9 degree-days) at the start of the first exposure period and either 2 or 3 d postfertilization (18 or 27 degree-days) at the start of the second exposure period. Six incubators were positioned at substrate depths of 0 cm (surface) or 20 cm below the substrate surface (hereafter 20 cm) in each plot (three incubators per substrate depth). Embryos during both periods were exposed to the same pellets, deposited on the substrate within the plots on September 12, 2018. Scuba divers evenly distributed pellets within each plot to achieve treatment densities of 28 kg/m² (heavy), 14 kg/m² (medium), 7.0 kg/m² (light), 3.5 kg/m² (xlight), and 1.75 kg/m² (xxlight) (Figure 3). Densities were based on initial, weighed quantities of pellets before deposition and dispersal on substrates within treatment plots. Control plots received no pellets.

During the first exposure period, we noted embryo mortality almost immediately following pellet treatment. However, we also noted higher than anticipated embryo mortalities at the substrate surface in control plots, probably due to exposure of the embryos to direct sunlight (incubators were placed uncovered on the substrate surface). We ended the first exposure at day 6 because many embryos in control plots remained alive, but nearly all embryos in plots treated with pellets (surface and at 20-cm depth) had died. Additional adult Lake Trout were spawned to obtain new embryos to use in a second exposure within the original pellet treatment plots (i.e., no new pellets were applied). In the second exposure, incubators placed at the substrate surface were covered by flat rocks to reduce sunlight exposure. Pellet densities were documented by time-series photography
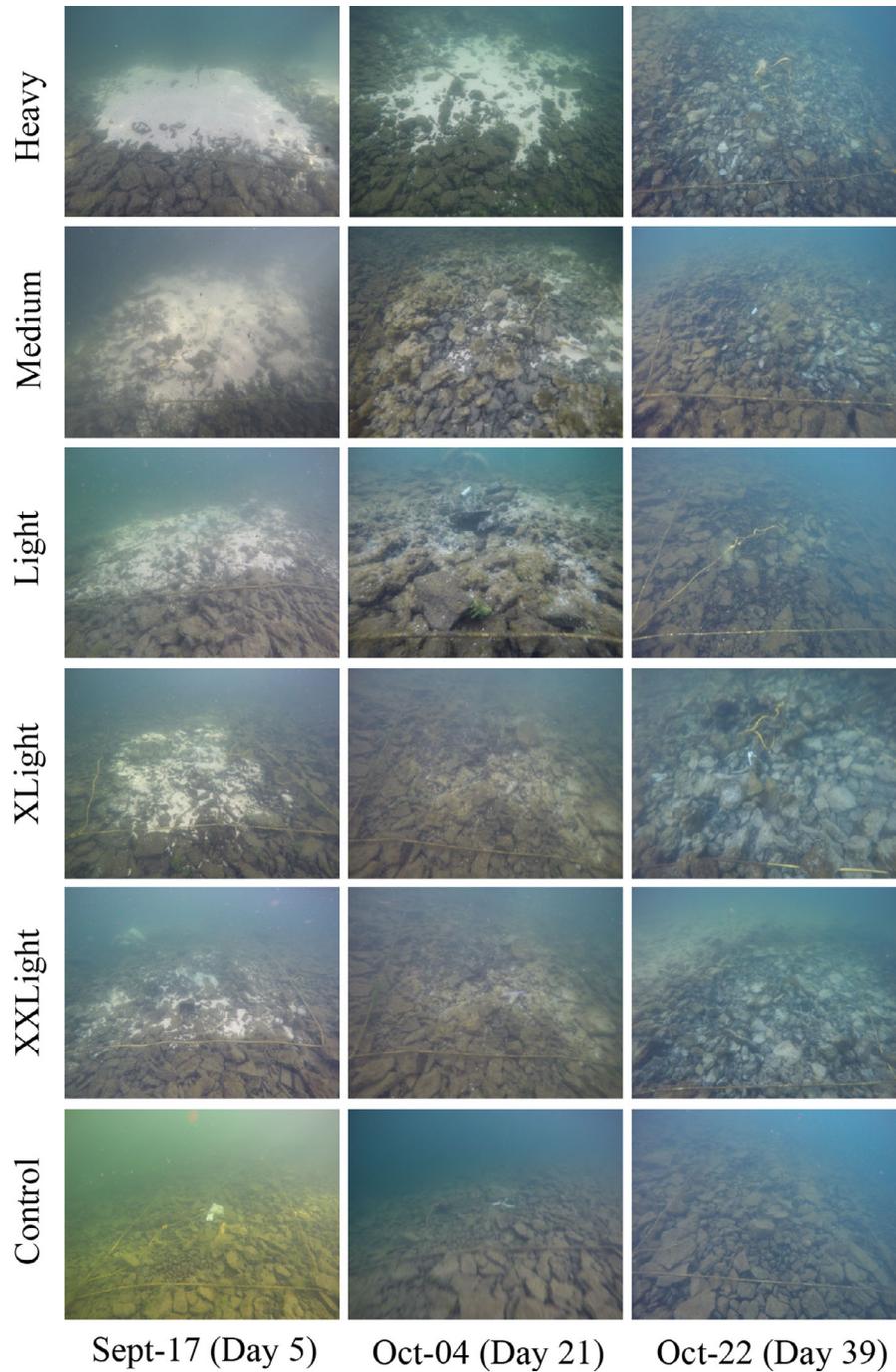
FIGURE 3. Recovery of the 9-m^2 plots treated on September 12, 2018, with pellets at densities of 28.0 kg/m^2 (heavy), 14.0 kg/m^2 (medium), 7.0 kg/m^2 (light), 3.5 kg/m^2 (xlight), 1.75 kg/m^2 (xxlight), and control (no pellets). Plots were delineated by yellow polypropylene rope. An embryo incubator and water quality logger are visible at the surface of the control plot on September 17.

(Figure 3) and observed to be lower than the densities initially applied due to 10 d of decomposition and dissipation of pellet material prior to the beginning of the second exposure period.

Data collection and analysis.— Dissolved oxygen concentrations (mg/L) and water temperatures (°C) were recorded using miniDOT loggers (Precision Measurement Engineering; 60-min sampling interval) placed at substrate

depths of 0 cm (surface) or 20 cm at the center of one of each of the five treatment replicates and one control replicate. Mean daily DO, coefficient of variation (CV; 100 SD/mean) of mean daily DO, and mean daily water temperature were calculated for the entirety of the experiment.

The number of live embryos in each incubator was recorded before and after exposures. Percent embryo mortality in each incubator was calculated by dividing the number of live embryos posttreatment by the number of live embryos pretreatment, subtracting the proportional survival from one to obtain the proportional mortality, and multiplying the proportional mortality by 100. Analysis of variance (ANOVA) was used to evaluate the effects of pellets, depth, and the interaction between pellets and depth on mean embryo mortality ($\alpha = 0.05$). The pellet factor had six levels (i.e., controls and the five treatments). The depth factor had two levels (i.e., 0 and 20 cm). The response variable was the mean percent embryo mortality of the three incubators at the same depth within each plot (experimental unit). Statistical assumptions of ANOVA were checked with residuals versus fitted values (homogeneity of variance) and quantile–quantile (normality) plots. Mean percent embryo mortality was logit transformed to increase normality of the data distribution and reduce heteroscedasticity of the group means (Sokal and Rohlf 1995). If a statistical difference among group means was detected, a post hoc Tukey's honestly significant difference multiple comparison procedure was used to test for differences between group means. A nonlinear model (similar to the von Bertalanffy growth model but with the asymptote fixed at 100%) was used to evaluate the relationship between the hours that DO was <3.4 mg/L and percent embryo mortality. Dissolved oxygen data from each of the six miniDOT loggers and the associated embryo mortalities were used in the model, with the upper 95% confidence limit truncated at 100 because the embryo mortalities were percentage data bound by 0 and 100. Data were manipulated using the "dplyr" package (Wickham et al. 2018) in Program R and analyzed using Program R (R Core Team 2018).

RESULTS

Dissolved Oxygen Concentration

Pellet treatments at the Thomas Bank spawning site reduced DO at the substrate surface and at 20 cm below the substrate surface (Figure 4). At the substrate surface, the DO of the heavy treatment declined most quickly and was 0.2 mg/L at 48 h following the placement of the pellets. Mean daily DO at the surface of the treatment plots during the first exposure period varied from 2.7 (heavy treatment) to 7.8 mg/L (xxlight; Table 1); values during the

second exposure period were similar, varying from 1.2 mg/L (heavy) to 8.0 mg/L (xxlight; Table 2). Variability of DO at the substrate surface was generally low in all pellet treatments except in the heavy treatments where CV = 120 during the first exposure and CV = 192 during the second exposure.

At a 20-cm depth within the substrate, the DO of all treatment plots declined to near zero within a few days of pellet treatment (Figure 4). With exception of the xxlight treatment, the DO then remained <2.3 mg/L at 20 cm through the end of the second exposure period (day 21). Mean DO during the first exposure period varied from 1.6 to 2.6 mg/L in xlight, light, medium, and heavy treatments and was lower during the second exposure, when it varied from 0.2 to 0.4 mg/L (Tables 1, 2). Variability of DO at a 20-cm depth within the substrate was generally high (CV > 100) during both exposure periods except in xxlight (both periods) and in the medium and heavy treatments (second exposure period only). The xlight, light, medium, and heavy treatments maintained lethal DO at the 20-cm depth for a month following application, despite dissolution and dissipation of pellets from the substrate surface (Figure 3).
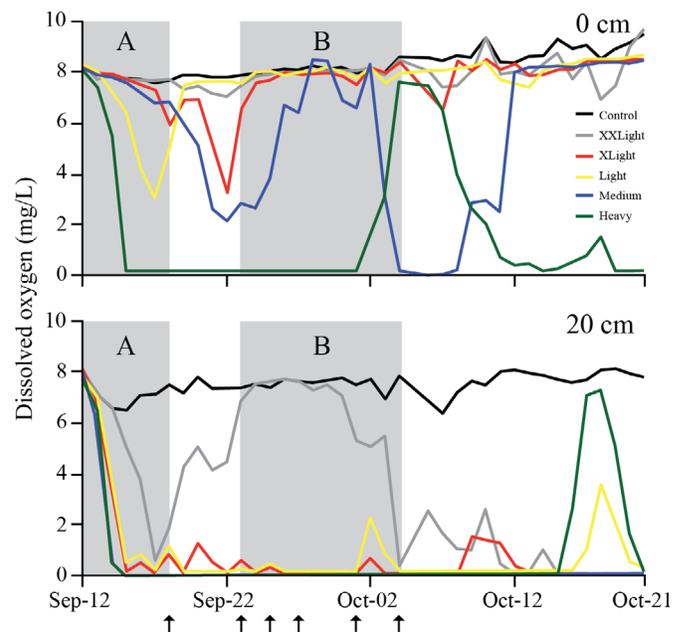

FIGURE 4. Mean daily dissolved oxygen concentrations (mg/L) in pellet treatment and control plots at the substrate surface (0 cm) and 20 cm below the surface during autumn 2018. The first Lake Trout embryo exposure period (shaded gray; A) occurred September 12–18 and the second exposure period (B) occurred September 23 to October 4. Pellets were applied to the treatment plots once on September 12 at densities of 1.75 kg/m² (xxlight), 3.5 kg/m² (xlight), 7.0 kg/m² (light), 14.0 kg/m² (medium), and 28.0 kg/m² (heavy). Black arrows indicate dates when pellets and substrate were disturbed by scuba divers to examine embryo incubators.

TABLE 1. Physiochemical characteristics, coefficient of variation (CV) of daily mean DO (%), mean number (SE in parentheses) of pretreatment, live Lake Trout embryos per treatment combination ($n = 3$), mean (SE in parentheses) Lake Trout embryo mortality (%), and mean (SE in parentheses) logit-transformed Lake Trout embryo mortality (%) of in situ organic pellet treatments at the Thomas Bank spawning site in Yellowstone Lake during the first exposure period (Figures 3, 4) on September 12–18, 2018. Pellets were applied to the substrate surface (0 cm) on September 12, 2018. In the last column, values marked with the same letter (x–z) are not statistically different in logit-transformed mortality among treatment types ($\alpha = 0.05$). Incubators at 0 cm during the first exposure period were uncovered and exposed to sunlight, resulting in high mortality (>75%) in the control and xxlight, xlight, light, and medium treatments despite low hours of DO < 3.4 mg/L.

Depth (cm)	Treatment type	Pellet biomass density (kg/m²)[a]	Mean temperature (°C)	Mean DO (mg/L)	Maximum DO (mg/L)	Minimum DO (mg/L)	Daily DO CV (%)	Hours DO < 3.4 mg/L	Live embryos	Mortality (%)	Mortality (logit; %)
0	Control	0.00	12.3	7.8	9.6	6.7	3	0	47 (1.2)	75 (2.1)	1.02 (0.10) y
	XXLight	1.75	12.3	7.8	9.1	6.5	3	0	49 (0.2)	97 (3.0)	3.04 (0.50) z
	XLight	3.50	12.3	7.4	8.5	0.1	10	3	49 (0.2)	99 (0.4)	3.44 (0.12) z
	Light	7.00	12.4	5.9	8.6	0.1	34	27	48 (0.3)	100 (0.0)	3.66 (0.00) z
	Medium	14.00	12.3	7.4	8.4	5.0	7	0	49 (0.0)	100 (0.0)	3.66 (0.00) z
	Heavy	28.00	12.4	2.7	8.4	0.2	120	104	49 (0.4)	100 (0.0)	3.66 (0.00) z
20	Control	0.00	12.3	7.0	8.1	5.3	6	0	49 (0.3)	39 (4.0)	−0.42 (0.16) x
	XXLight	1.75	12.3	4.4	8.5	0.0	60	52	49 (0.6)	97 (2.2)	3.00 (0.41) z
	XLight	3.50	12.3	2.4	8.2	0.0	120	112	48 (2.0)	100 (0.0)	3.66 (0.00) z
	Light	7.00	12.4	2.6	8.1	0.2	107	105	50 (0.0)	100 (0.0)	3.66 (0.00) z
	Medium	14.00	12.4	1.6	8.4	0.0	165	126	48 (0.4)	100 (0.0)	3.66 (0.00) z
	Heavy	28.00	12.4	1.6	8.4	0.0	161	125	48 (1.3)	100 (0.0)	3.66 (0.00) z

[a]Pellet quantities were measured prior to the divers depositing the pellets on the substrate, and densities were observed to decline throughout the study period (Figure 3).

TABLE 2. Physiochemical characteristics, CV of daily mean DO (%), mean number (SE in parentheses) of pretreatment, live Lake Trout embryos per treatment combination ($n = 3$), mean (SE in parentheses) Lake Trout embryo mortality (%), and mean (SE in parentheses) logit-transformed Lake Trout embryo mortality (%) of in situ organic pellet treatments at the Thomas Bank spawning site in Yellowstone Lake during the second exposure period (Figures 3, 4) from September 23 to October 4, 2018. Pellets were applied to the substrate surface (0 cm) on September 12, 2018. In the last column, values marked with the same letter (w–z) indicate no statistical difference in logit-transformed mortality among treatment types ($\alpha = 0.05$). Incubators at 0 cm during the second exposure period were covered and protected from sunlight.

Depth (cm)	Treatment type	Pellet biomass density (kg/m²)[a]	Mean temperature (°C)	Mean DO (mg/L)	Maximum DO (mg/L)	Minimum DO (mg/L)	Daily DO CV (%)	Hours DO < 3.4 mg/L	Live embryos	Mortality (%)	Mortality (logit)
0	Control	0.00	10.3	8.1	10.7	5.9	2	0	49 (0.1)	13 (1.0)	−1.72 (0.08) w
	XXLight	1.75	10.3	8.0	11.5	5.7	3	0	50 (0.0)	26 (1.6)	−1.01 (0.08) wx
	XLight	3.50	10.3	7.8	10.8	0.5	6	4	49 (0.2)	58 (18.9)	0.56 (0.99) wxy
	Light	7.00	10.3	7.9	12.4	4.4	3	0	49 (0.1)	45 (10.7)	−0.22 (0.43) wx
	Medium	14.00	10.3	5.4	22.7	0.0	52	130	49 (0.1)	95 (4.9)	2.98 (0.68) yz
	Heavy	28.00	10.5	1.2	8.3	0.2	192	259	50 (0.0)	100 (0.0)	3.66 (0.00) z
20	Control	0.00	10.3	7.6	8.4	4.5	3	0	49 (0.5)	12 (1.2)	−1.82 (0.10) w
	XXLight	1.75	10.3	6.3	8.1	0.0	34	32	50 (0.0)	78 (12.7)	1.62 (0.95) xyz
	XLight	3.50	10.4	0.2	3.9	0.0	134	299	49 (0.1)	99 (0.4)	3.45 (0.12) z
	Light	7.00	10.4	0.4	6.5	0.2	142	288	50 (0.0)	91 (8.9)	2.76 (0.90) yz
	Medium	14.00	10.7	0.0	0.0	0.0	3	300	49 (0.1)	100 (0.0)	3.66 (0.00) z
	Heavy	28.00	10.7	0.0	0.2	0.0	9	300	49 (0.2)	100 (0.0)	3.66 (0.00) z

[a]Pellet quantities were measured prior to the divers depositing the pellets on the substrate, and densities were observed to decline throughout the study period (Figure 3). Pellets had decomposed and dissipated into the lake water column for 10 d prior to the beginning of the second exposure.

Embryo Mortalities

Mean mortalities of embryos in the controls during the first exposure period were $75 \pm 2.1\%$ (mean $\pm$ SE) at the surface and $39 \pm 4.0\%$ at 20 cm, and both were statistically lower than in all pellet treatments ($P < 0.05$, Table 1; Figure 5A). Mean mortalities of embryos exposed to pellets during the first exposure period were high at all treatment levels at both the surface and at 20 cm, varying from $97 \pm 3.0\%$ to $100 \pm 0.0\%$ (Table 1; Figure 5A). Embryo mortality differed between the surface and 20-cm controls ($P < 0.05$) but not among the treatments ($P > 0.05$), resulting in an interaction (ANOVA: $F_{2,\ 36} = 4.771$, $P = 0.004$; Table 1; Figure 5A).

Mean mortalities of embryos in the controls during the second exposure period were $13 \pm 1.0\%$ at the surface and $12 \pm 1.2\%$ at 20 cm, and both were lower than mortalities of embryos in all pellet treatments ($P < 0.05$) except xxlight at the surface ($P > 0.05$, Table 2; Figure 5B). Mean mortalities of embryos exposed to pellets during the

second exposure period were high at all treatment levels at 20 cm, varying from $78 \pm 12.7\%$ to $100 \pm 0.0\%$ (Table 2; Figure 5B). Mean mortalities of embryos at the surface were high in the medium and heavy treatments, at $95 \pm 4.9\%$ and $100 \pm 0.0\%$, respectively. Mean mortalities of embryos at the surface and 20 cm were similar within the control, medium, and heavy treatments ($P > 0.05$); however, mortalities of embryos were higher at the surface than at 20 cm in the xxlight, xlight, and light treatments ($P < 0.05$), resulting in an interaction (ANOVA: $F_{2,\ 36} = 3.838$, $P = 0.011$; Table 2; Figure 5B).

Minimum Pellet Biomass and Exposure Duration

An application density of 1.75 kg of pellets/m^2 (xxlight) resulted in 97% mortality at the surface and at 20 cm immediately following spawning site treatment (first exposure period; Table 1; Figure 5A). Mortalities in the control plots were higher than expected, suggesting that handling of embryos (i.e., exposure to sunlight) also caused some of the embryo mortalities during the first exposure. The potency of pellets may have declined over time. Ten days after application, 3.50 kg of pellets/m^2 (xlight) was required to achieve near 100% mortality at the 20-cm depth, with much higher application densities required at the surface (second exposure period; Table 2; Figure 5B). Mortality of Lake Trout embryos rapidly increased as hours of exposure to DO < 3.4 mg/L increased (Tables 1, 2). Parameter estimates for the model were $K = 0.02$ (95% CI $= 0.02$–0.03) and $t_0 = -10.02$ (95% CI $= -15.78$ to -6.05). Percent embryo mortality increased exponentially
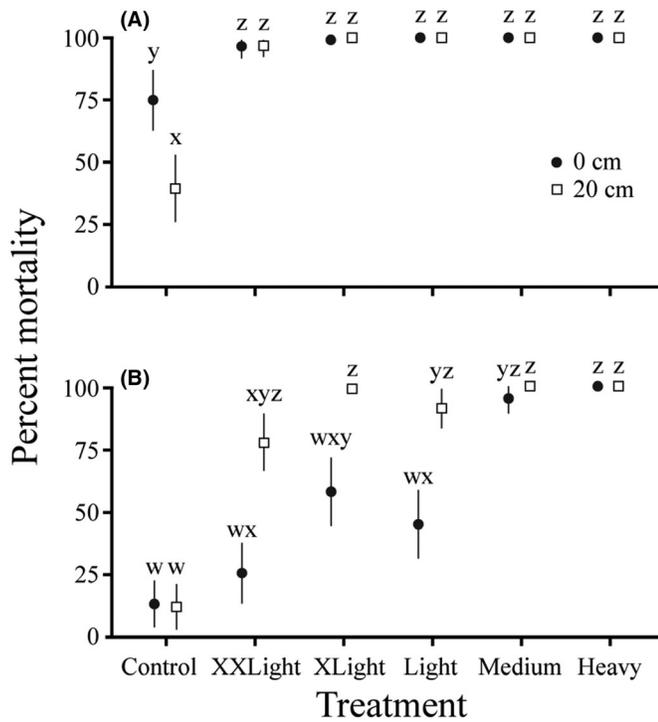

FIGURE 5. Mean percent mortality (error bars show 95% CIs) of Lake Trout embryos at 0 cm (substrate surface; black circles) and 20 cm below the substrate surface (open squares) in control plots and pellet treatment plots during **(A)** the first exposure period (September 12–18) and **(B)** the second exposure period (September 23 to October 4) at the Thomas Bank spawning site on Yellowstone Lake in 2018. Pellets were applied to the treatment plots once on September 12 at densities of 1.75 kg/m^2 (xxlight), 3.5 kg/m^2 (xlight), 7.0 kg/m^2 (light), 14.0 kg/m^2 (medium), and 28.0 kg/m^2 (heavy). Measurements marked with the same letters (w–z) within each exposure period indicate no statistical difference in logit-transformed mortality among treatment types ($\alpha = 0.05$)
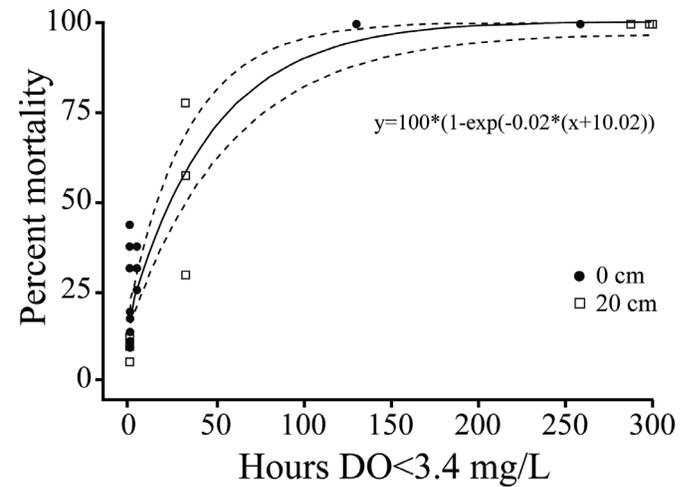

FIGURE 6. Mean percent mortality of Lake Trout embryos at 0 cm (substrate surface; black circles; $n = 18$) and 20 cm below the substrate surface (open squares; $n = 18$) after exposure to increasing hours of lethal DO concentrations (< 3.4 mg/L) during the second exposure period (September 23 to October 4) at the Thomas Bank spawning site on Yellowstone Lake. The nonlinear model has the asymptote fixed at 100% (the dashed lines show the 95% CI).

at a decreasing rate as the total number of hours that DO was <3.4 mg/L increased, with a minimum exposure period of about 200 h required to ensure complete mortality (Figure 6).

DISCUSSION

Organic pellets caused high (up to 100%) mortality of Lake Trout embryos during in situ experiments at the Thomas Bank spawning site in Yellowstone Lake, even in treatments with the lightest pellet application densities (1.75 kg/m^2). Mortalities in all treatments were high at a 20-cm depth in the substrate, where Lake Trout embryos would naturally settle within spawning habitats that have interstices of this depth.

Pellets remained on treated substrates for several weeks and overcame many of the shortcomings of using Lake Trout carcasses noted previously. Pellets did not visibly float or drift from treatment areas and did not attract bears or piscivorous waterbirds that (particularly at shallow spawning sites) depleted and dispersed carcass material (Poole 2019; Thomas et al. 2019). No wildlife were observed feeding on or being otherwise attracted to the pellets. Exposure of embryos during two periods (spanning 21 d) allowed assessment of the longevity and potency of the pellets for inducing mortality on spawning sites. Pellets remained visible at all treatment densities on day 4 after placement, forming a paste that adhered to the substrate, penetrated into interstices, and prevented drift. By day 21, at the end of the second exposure period, many pellets had dissolved and whole pellets were only evident in the medium and heavy treatment plots. Although pellet material remained visible through the end of the study on day 39, the plots had then largely recovered from the treatments.

Decomposition of organic pellets increased the biological oxygen demand and greatly reduced DO at spawning sites, but DO varied substantially and unexpectedly at both the surface and at 20 cm in treated plots. We had expected that variation of DO would be greater at the substrate surface than at 20 cm because of the closer proximity to and exchange of oxygenated lake water. We surmise that variation in DO was largely a result of current-driven water exchange. Substrate characteristics of Lake Trout spawning substrates in Yellowstone Lake allow sufficient water exchange to maintain ambient DO, even when covered with gas-impermeable tarps (e.g., Carrington Island; Thomas et al. 2019). However, the routine removal of incubators by scuba divers to monitor the mortality of embryos may have affected DO levels. These removals, while brief (< 5 min per plot), necessarily disturbed the substrate, suspended pellet material above treatment plots, and potentially allowed oxygenated water to penetrate into interstices.

Low DO appeared to be the primary cause of Lake Trout embryo mortality at Thomas Bank. During the second exposure, nearly complete mortality occurred in pellet treatment plots that achieved more than 200 h of DO < 3.4 mg/L. High mortality also occurred in plots where DO levels were lethal for fewer hours, suggesting that other factors (e.g., sunlight, discussed above) contributed to embryo mortality. Pellets resulted in elevated levels of ammonia and hydrogen sulfide in laboratory trials (Poole 2019). Decomposition of organic material also increases carbon dioxide concentrations and fungal proliferation (Sly 1988), which may have influenced mortality, particularly during the second exposure because embryos were added to existing pellet material that had already undergone decay. In large-scale spawning site applications, maintenance of DO below the 3.4 mg/L threshold for at least 200 h should be a reliable predictor of embryo mortality.

Treatment of spawning sites with pellets at a large scale will be required to reduce embryo survival of Lake Trout in Yellowstone Lake to mimic low survival in degraded habitats in their native range. Large-scale field application of pellets should be more efficient than application of carcass material because no processing is required during deposition and pellets can be distributed mechanically. A pellet application density of 1.75 kg/m^2 is expected to achieve >75% embryo mortality of treated areas at a 20-cm depth within substrate interstices. Pellet decomposition rates may be slower at spawning sites in deeper water, due to lower temperatures or reduced light, potentially reducing treatment effectiveness. Additionally, the effect of pellet decomposition on Lake Trout embryos that settled deeper than 20 cm in the substrate interstices was not documented; however, substrate interstices observed by divers when delineating Lake Trout spawning sites in Yellowstone Lake were generally ≤20 cm deep. Treating the entire spawning substrate at Thomas Bank (0.71 ha) at a density of 1.75 kg/m^2 would require 12,425 kg of pellets at a cost of US$15,000. Treating all of the delineated (11.4 ha) spawning habitat in Yellowstone Lake would require 199,500 kg of pellets at a cost of $250,000 and could ultimately reduce the $2 million spent annually on the current gillnetting suppression program on Yellowstone Lake. Treatments at these large scales would require application by helicopter or by broadcast spreader mounted on the back of a boat, similar to how granulated lampricide is distributed (Dawson and Kolar 2003) or how feed is distributed for intensive aquaculture operations (Goddard 1996). Treatment coverage could be tracked with onboard GPS-equipped chartplotter units to ensure that appropriate densities of pellets are delivered.

The use of pellets to treat spawning sites may result in unintended consequences. For example, pellets may increase nutrients such as nitrogen (N), which could affect

periphyton, macrophytes, and macroinvertebrates at a local scale. However, because the surface area of known spawning sites (11.4 ha) represents only 0.03% of the total surface area (36,017 ha) of Yellowstone Lake, adverse ecosystem-scale effects would be unlikely. Pellets would be applied in autumn, allowing the winter for any remaining material to break down and nutrients to disperse before higher temperatures and sunlight return the following summer. No short-term effects on productivity were detected after carcass treatments (Thomas 2017), but any effects of large-scale pellet additions (3.6% N) could be countered by removal of gillnetted Lake Trout carcasses (~11% N).

Treatment of spawning sites might also force site avoidance, straying, and pioneering of new spawning sites by Lake Trout in Yellowstone Lake. Given that Lake Trout have been present in Yellowstone Lake for at least 30 years, we assume that a majority of the highest-quality spawning habitat is being used and has been discovered. Any newly pioneered sites would probably be of lower habitat quality and therefore result in lower survival, which would benefit suppression efforts. Moreover, the intense gillnetting of spawning sites during autumn to kill adult Lake Trout has probably already led to widespread straying over the past two decades; the degree to which pellet treatments would enhance this is unknown. Tagging and acoustic telemetry of adult fish (Williams 2019) will continue to ensure detection of any pioneering and establishment of new spawning sites.

Inference of our experimental results is limited to one Yellowstone Lake spawning site during one year. However, our work builds upon a body of evidence generated during previous studies of the effects of carcass and pellet decomposition on embryo mortality (Poole 2019; Thomas et al. 2019). We therefore suggest that the use of pellets to reduce Lake Trout embryo survival has implications for native fish conservation and sport fish restoration beyond Yellowstone National Park. Lake Trout have been widely introduced to lakes and reservoirs in the western United States, where they now occur in more than 200 waters following intentional or illegal introductions or invasive movements among lakes via river networks (Martinez et al. 2009). In Montana, introduced Lake Trout invaded Flathead Lake (Spencer et al. 1991) and the Flathead River system, including headwater lakes in Glacier National Park (Fredenberg 2002), and Swan Lake (Cox 2010). Lake Trout have also expanded throughout Lake Pend Oreille, Idaho (Hansen et al. 2010). Lake Trout suppression by gillnetting in these systems (Syslo et al. 2013; Hansen et al. 2016; Fredenberg et al. 2017) could be enhanced by use of pellets to induce embryo mortality because spawning sites are known in many of these lakes (e.g., Dux et al. 2011). Moreover, other predatory fish such as Channel Catfish *Ictalurus punctatus*, Largemouth

Bass *Micropterus salmoides*, Smallmouth Bass *Micropterus dolomieu*, and Walleye *Sander vitreus* have been introduced to the western United States and are negatively influencing native fishes and the ecosystems they support (McMahon and Bennett 1996; Fritts and Pearsons 2004; Olden and Poff 2005; Schade and Bonar 2005; Sanderson et al. 2009; Rahel and Smith 2018). Managers have implemented (or are contemplating) suppression programs for invading fishes to preserve or restore sympatric native fishes or desired sport fish populations, or both (Coggins et al. 2011). We suspect that spawning sites of these invasive species and others in which embryos incubate on or within the substrate could be temporarily degraded by pellet treatments. Effects may be even more pronounced in waters with higher temperatures or longer photic periods than Yellowstone Lake. Additional laboratory and in situ experiments should be conducted to assess the efficacy of pellets for suppression of other invasive fish species in other locations.

An integrated pest management (IPM) approach uses all suitable techniques in a compatible manner to maintain pest population levels below those causing harm (FAO 1968; Dent 1995). By incorporating a variety of suppression methods to target multiple life stages of an invasive species (Ehler 2006), the IPM approach has been most widely used in terrestrial systems for the control of agricultural crop pests (Flint and Van den Bosch 1981; Peshin et al. 2009). The suppression of Sea Lamprey *Petromyzon marinus* in the Laurentian Great Lakes, however, is an example of IPM in which chemical treatments, pheromone attractants, migration barriers, and other methods are being used in combination to successfully control an aquatic invasive species (Sawyer 1980; Christie and Goddard 2003; Johnson et al. 2009). We think the Yellowstone Cutthroat Trout conservation program on Yellowstone Lake will similarly benefit from an IPM approach wherein complementary methods are used to reduce Lake Trout recruitment and efficiently maintain a suppressed Lake Trout population over the coming decades.

Conclusions

Gill netting effectively curtails Lake Trout population growth and allows Yellowstone Cutthroat Trout recovery in Yellowstone Lake (Koel et al. 2019), but alternative methods are needed to maintain Lake Trout suppression during the coming decades at a reduced cost, with limited Yellowstone Cutthroat Trout bycatch and with fewer influences on visitors (e.g., wilderness experience) and native wildlife. Relative to the expansive lake areas intensively gillnetted over a 6-month season (> 60 km of gill nets set daily), methods to suppress Lake Trout embryos allow targeting of small, well-delineated sites during a period of 2–3 weeks in autumn where a majority of the year-class is concentrated. Decomposition of Lake Trout

carcasses (whole or ground) and organic pellets cause lethal levels of DO and induce high mortality of embryos. Both materials dissipate and allow site recovery for unimpeded Lake Trout spawning the following year. However, plant-based organic pellets generally caused DO to decline below lethal levels faster (within 2 d) than carcass material and formed a paste that adhered to and within the substrate and therefore maintained a longer period of potency for eliciting mortality of embryos. In addition, pellets can be produced in advance of the treatment period, stored in sufficient quantities, transported easily, and applied over large spawning habitats with precision. A combination of Lake Trout carcass and pellet materials will therefore probably be used to treat spawning sites in Yellowstone Lake. These methods may also be used in an IPM approach in other systems for targeting invasive freshwater fish that deposit gametes in or on substrates.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.

Appendix: Lake Trout Spawning Sites in Yellowstone Lake

TABLE A.1. Lake Trout spawning sites in Yellowstone Lake located by gillnetting and acoustic telemetry and verified by the presence of embryos. Embryos were found by snorkeling, scuba diving, remotely operated vehicle, or benthic sled sampling. Map locations refer to Figure 1. Abbreviations are as follows: UTM = Universal Transverse Mercator.

Spawning site and total	Map location	Year located	Surface area (ha)	Depth range (m)	Substrate type	UTM coordinates (north, east)
Carrington Island	1	1996	0.5	0–4	Cobble	535422, 4922626
Joe's Hump	2	2003		9–24	Thermal	534271, 4919596
West Thumb Geyser	3	2008	1.8	5–10	Thermal	534448, 4918621
Solution Hump	4	2000	1.3	15–20	Thermal	539088, 4919480
Breeze Channel[a]	5	2006	1.1	26–29	Thermal	540846, 4921338
Wolf Point	6	2008		15–20	Cobble	546871, 4918651
Snipe Point[a]	7	2006	2.0	1–20	Cobble	548357, 4917657
South Frank Hump[a]	8	2008	1.1	10–30	Thermal	552923, 4916827
Olson Reef	9	2007	0.3	13–20	Cobble	549276, 4915796
Flat Mountain Hump	10	2007	1.1	9–28	Bedrock	549105, 4914781
Flat Mountain Elbow[a]	11	2008	0.6	1–10	Cobble	549666, 4914566
Flat Mountain Mid[a]	12	2015	0.9	1–5	Cobble	547933, 4913593
Thomas Bank	13	2015	0.7	1–15	Cobble	546325, 4913384
South Arm Mid	14	2017		10–20	Cobble	551864, 4911198
Total			11.4			

[a]Lake Trout spawning sites where embryos were found using a benthic sled by Simard (2017).