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ARTICLE

Evaluation of Remote Site Incubators to Incubate Wild- and Hatchery-Origin Westslope Cutthroat Trout Embryos

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Abstract
Fish managers must weigh trade-offs among cost, speed, efficiency, and ecological adaptation when deciding how to translocate native salmonids to either establish or genetically augment populations. Remote site incubators (RSIs) appear to be a reasonable strategy, but large-scale evaluations of this method have been limited. We used 129 RSIs to incubate >35,700 eyed embryos of Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* at eight sites within the upper 30 km of the Cherry Creek basin (Madison River, Montana) from 2007 to 2010, after using piscicides to remove all fish. We obtained gametes from 258 parental-pair crosses (164 females and 258 males) from four wild populations and two hatchery broods. All embryos were incubated to the eyed stage in two hatcheries prior to placing them in RSIs. Green-to-eyed egg survivals were higher for progeny of wild-spawned adults (median, 91.0%; 95% CI, 88.7–93.7%) than for progeny of hatchery-spawned adults (median, 81.7%; 95% CI, 74.9–88.4%), and this difference was highly significant ($P < 0.01$). Over 26,500 fry were counted leaving RSIs. Median embryo-to-fry survival was 75.6% (95% CI, 72.2–79.0%). Fry

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exited individual RSIs from 8 to 45 d after embryo translocation. Fry survivals differed among years and sites, and year was more important than site in explaining variation in survival. The success of RSI fry introductions was confirmed by annual monitoring of fish abundance, which indicated that abundances of Westslope Cutthroat Trout 5 to 9 years after RSI introductions were equal to or higher than abundances of nonnative salmonids prior to their removal using piscicides.

Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* were formerly the most widely distributed subspecies of inland Cutthroat Trout *Oncorhynchus clarkii*, inhabiting both sides of the Continental Divide across the northern Rocky Mountains in the United States, including parts of Montana, Idaho, Oregon, Washington, and the provinces of Alberta and British Columbia in Canada (Behnke 1992; Shepard et al. 2005). Their distribution and abundance have been significantly reduced in the last 100 years by hybridization and competition with nonnative species, habitat loss, and overharvest (Liknes and Graham 1988; Behnke 1992; Shepard et al. 1997, 2005).

Translocation is a potential conservation tool for restoring populations of native species into suitable habitats within their historical range (Griffith et al. 1989; Minckley 1995; George et al. 2009) and is one strategy managers are using to conserve Westslope Cutthroat Trout (Shepard et al. 2005; Montana Department of Fish, Wildlife and Parks 2007). Many state and federal agencies, a private landowner, and Montana State University collaborated to translocate Westslope Cutthroat Trout into Cherry Creek, a major tributary to the Madison River in the Missouri drainage in southwestern Montana, after removing nonnative fish using piscicides (Clancey et al. 2019).

A major consideration was the method used to translocate Westslope Cutthroat Trout from several different extant populations located within the upper Missouri River basin. We needed to reduce risks of transferring fish pathogens or parasites as much as possible. This severely restricted our ability to transfer fry or older individuals from wild populations into state or federal hatcheries or directly into Cherry Creek. Translocating fertilized eggs reduces these risks because many disease organisms are not transmitted vertically, and fertilized eggs can be disinfected with an iodophor solution during and after the water-hardening phase (Bullock and Stuckey 1987; Yoshimizu et al. 1989; Pravecek and Barnes 2003). Incubating embryos in remote site incubators (RSIs) located throughout the upper basin appeared to offer the best alternative because translocating fertilized eggs reduces pathogen and parasite risks, fry emergence success could be quantified, and RSIs allow fry to emerge and disperse more naturally, which may promote natal imprinting and local selection (Donaghy and Verspoor 2000; Kaeding and Boltz 2004).

Our research question was, “Can RSIs be used to establish a genetically diverse population of Westslope Cutthroat Trout that preserves some of the genetic legacy of Westslope Cutthroat Trout found in the upper Missouri River basin?” Our specific objectives were to determine if (1) green-to-eyed egg survivals of progeny from wild-spawned and hatchery-spawned adults differed, (2) survivals of green-to-eyed eggs differed among years, (3) survivals of green-to-eyed eggs differed among individual donor stocks, (4) survivals of eyed embryos to emergent fry within RSIs differed among years, (5) survivals of eyed embryos to emergent fry within RSIs differed among release sites, and (6) survivals of eyed embryo to fry within RSIs were influenced by water temperatures.

METHODS

Study Area

Cherry Creek originates in the Madison Mountain Range of Montana at an elevation of 2,652 m and flows northeast for 37.5 km before joining the Madison River (Missouri River basin) at an elevation of 1,350 m (Figure 1). Although the Cherry Creek basin lies within the historical range of Westslope Cutthroat Trout, an 8-m-high waterfall located about 13 km upstream from its confluence with the Madison River probably resulted in the stream being historically barren of fish above this barrier. Nonnative Rainbow Trout *O. mykiss*, Yellowstone Cutthroat Trout *O. clarkii bouvieri*, and Brook Trout *Salvelinus fontinalis* were stocked into the upper basin during the early 1900s. These nonnative species were eradicated using piscicides from 2003 to 2010 (Clancey et al. 2019).

Donor Populations

To address objectives 1 and 3, we collected gametes from four wild populations (Muskrat, Ray, White’s, and Bray’s Canyon creeks) and two hatchery populations (Sun Ranch and Washoe Park; Clancey et al. 2019). These wild Westslope Cutthroat Trout populations were selected as donors because they were in the upper Missouri River drainage, were genetically pure, and could provide

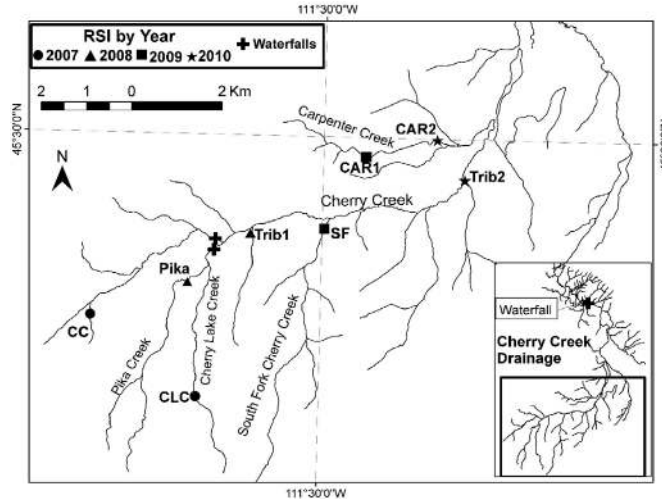

FIGURE 1. Map of the upper Cherry Creek study area, showing the eight locations of remote site incubators (RSIs) by year of eyed embryo introduction and the locations of waterfalls that prevented upstream fish movement. The inset map shows the entire Cherry Creek basin and the restoration project area above a waterfall, with the upper study area outlined with a bold rectangle. Release site locations are upper Carpenter Creek (CAR1), lower Carpenter Creek (CAR2), upper Cherry Creek (CC), upper Cherry Lake Creek (CLC), lower Pika Creek (Pika), lower South Fork Cherry Creek (SF), a tributary below the mouth of Cherry Lake Creek (Trib1), and a tributary just below Sweden Creek (Trib2).

sufficient eggs for translocation without unacceptable effects to the source populations (Dunham et al. 2011).

The Sun Ranch Hatchery brood is composed of Westslope Cutthroat Trout from several extant Westslope Cutthroat Trout populations located within the upper Missouri River basin, including Muskrat, White's, and Ray creeks. Washoe Park Hatchery is the state of Montana's primary Westslope Cutthroat Trout conservation hatchery, with brood fish originating from 16 extant populations west of the Continental Divide in Montana. Substantial genetic variation was found among the two hatchery populations and three of the wild populations (Andrews et al. 2016); comparable genetic information was unavailable for the Bray's Canyon Creek population.

Egg Collection

We spawned wild adults from Ray, Muskrat, and White's creeks during June and early July from 2007 to 2009 and from Bray's Canyon Creek in early July 2010. Captured adults were held in perforated plastic containers within their respective streams near the spawning areas until their gametes ripened. Eggs and sperm were stripped from ripe adults. Green (unfertilized) eggs obtained from each spawned female were divided into two equal lots and fertilized with sperm from two different males from the same source stock (hereafter, "stock"). Fertilized embryos from wild populations were water-hardened for 30 to 60 min in an iodophor–water solution (5 mL/L; Pravecek and Barnes 2003) to disinfect them prior to transport.

Because we were uncertain if we would have enough fry to release into our project area from wild-spawned fish, some progeny from Ray, Muskrat, and White's creeks were raised to maturity in a brood pond at the Sun Ranch Hatchery to produce supplemental embryos. Females raised in this pond reached larger sizes and had more eggs than wild-spawned females (Downs et al. 1997; Meyer et al. 2003; Kaeding and Koel 2011; Table 1).

Mature Westslope Cutthroat Trout in the Sun Ranch pond ripened and were spawned earlier than wild adults because water temperatures in the brood pond were warmer than in streams that supported wild donors. To time the release of Sun Ranch eyed embryos into RSIs closer to that of the wild-spawned embryos, we chilled the water used for incubating Sun Ranch embryos to slow their development. At the Washoe Park Hatchery, we collected gametes from ripe adults once a week. We used the same protocol for fertilizing hatchery eggs (half of each female's eggs fertilized with sperm from a different male) as we used for wild eggs. Wild-origin and Sun Ranch–origin fertilized embryos were incubated in vertical trays at Sun Ranch Hatchery, and Washoe Park embryos were incubated in incubation jars at Washoe Park Hatchery.

We used 258 parental pair crosses (164 females and 258 males) from the four wild populations and two hatchery broods (Table 1). Embryos from each pair mating (hereafter, "egg lot") represented a unique family group and were incubated until they reached the eyed stage. We removed and counted all eyed embryos from each egg lot before transporting them for release in RSIs.

Remote Site Incubators

Each RSI cost about US$150 and consisted of a 19-L (5-gal) black plastic bucket fitted with a lid and an inlet pipe that provided a minimum of 30 cm of hydraulic head (Kaeding and Boltz 2004; Rupert et al. 2007; Clancey et al. 2019; Figure 2). The pipe entered the RSI bucket near its bottom to percolate water up through the bucket. The internal components of the RSI included a wire-mesh basket that contained a layer of gravel on which eggs were placed and a layer of neutrally buoyant biomedia (TALOX plastic tower-packing saddles; www.koch-glitsch.com) that covered the eggs. A black lid was placed on the RSI unit to prevent direct sunlight from affecting the embryos. An outlet hole in the side of the bucket located just below the 30-cm water surface created by the hydraulic head allowed fry to exit the bucket of their own volition, usually after they absorbed their yolk sac. We attached an outlet pipe to this outlet hole and piped the water into a second capture bucket with fine mesh or small holes drilled to allow water, but not fry, to flow out.

TABLE 1. Total length of adult females (mm), number of Westslope Cutthroat Trout egg lots, and number of eyed embryos that were placed into remote site incubators (RSIs) by donor stock (W = wild, H = hatchery) and year, and the number of fry that emerged from RSIs by site and year in the upper Cherry Creek basin from 2007 to 2010. See Figure 1 for site abbreviations.

Donor stock, site, and total	Female length range (mm)	Year 2007	2008	2009	2010	Total
Number of egg lots						
Donor stock						
Bray's (W)	153–199				7	7
Muskrat (W)	158–270	22	27	24		73
Ray (W)	171–240	25	23	20		68
Sun Ranch (H)	381–438	13	13		3	29
Washoe Park (H)	191–361	21[a]	21[b]	12	2	56
White's (W)	164–213	8	9	8		25
Total		89	93	64	12	258
Number of eyed embryos						
Donor stock						
Bray's (W)	153–199				1,066	1,066
Muskrat (W)	158–270	5,445	3,204	4,004		12,653
Ray (W)	171–240	3,467	1,700	1,911		7,078
Sun Ranch (H)	381–438	3,075	3,209		398	6,682
Washoe Park (H)	191–361	1,121	2,645	1,714	154	5,634
White's (W)	164–213	1,015	974	636		2,625
Total		14,125	11,732	8,265	1,618	35,738
Number of fry						
RSI site						
CAR2					500	500
CC		5,476				5,476
CLC		5,231				5,231
Pika			4,356			4,356
SF				2,812		2,812
Trib1			4,713			4,713
Trib2					583	583
Total		10,707	9,069	5,645	1,083	26,504

[a]There were 21 parental pairs that were combined into three eggs lots and released into RSIs in7 both upper Cherry Lake Creek and upper Cherry Creek.
[b]For this value, 17 of these 21 egg lots were split and released into RSIs in both Pika Creek and the unnamed tributary just below the mouth of Cherry Lake Creek.

We counted all fry that moved to this capture bucket from the RSI prior to releasing them in the stream at each site (Figure 2).

Embryo Translocations

From 2007 to 2010, we translocated over 35,700 Westslope Cutthroat Trout eyed embryos into 129 RSIs at eight sites throughout the upper basin (Table 1). The number of embryos introduced from each donor population varied depending on their availability. All embryos from a single egg lot were placed together into one RSI, except for a few egg lots from Washoe Park and Sun Ranch, which were split among several RSIs. Embryos from Washoe Park were combined with other stocks in 62 of the 129 RSIs, including in at least one RSI at each site. The remaining 67 RSIs received embryos from a single wild donor stock, although multiple egg lots from this single stock went into some of these RSIs. The two egg lots from each female were placed into different RSI sites each year. We monitored each RSI every 2 to 3 d until we found no fry in its capture bucket for six consecutive days. We ensured that RSIs were supplying embryos with fresh water by maintaining the 30-cm water depth within the RSI, and we counted and released fry from capture buckets.

Survival Estimation

Green eggs to eyed eggs.— To evaluate objectives 1, 2, and 3, we calculated survival from the green egg to eyed egg stage for each egg lot by dividing the number of eggs

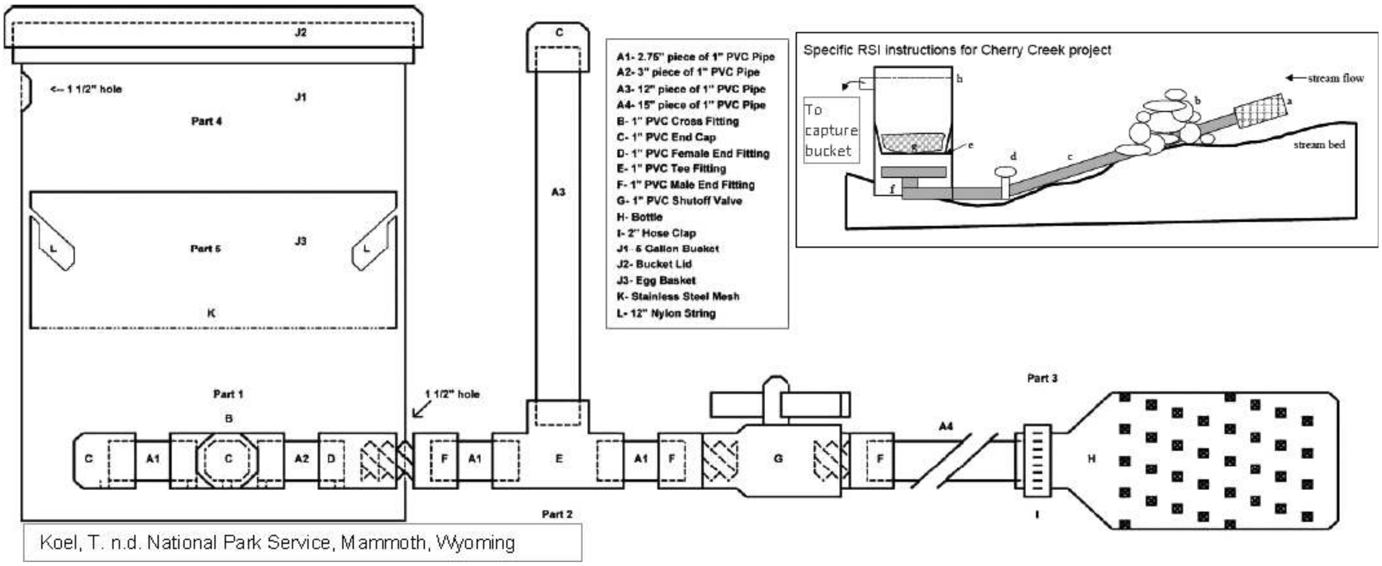

FIGURE 2. Cross section of a remote site incubator (RSI) used to introduce Westslope Cutthroat Trout embryos into eight locations within the Cherry Creek basin, showing construction details. The inset shows how an RSI was typically installed in the stream.

that survived to the eyed stage by the number of green eggs that were fertilized. Survivals for a few of the crosses were zero and green eggs were uncounted for some Washoe pairs, so these were excluded from further analyses. This left a total of 244 of a possible 258 egg lots available for analysis.

Eyed embryo to fry.— To evaluate objectives 4, 5, and 6, we estimated eyed embryo to fry survival (hereafter, "fry survival") for each RSI as the number of live fry released divided by the total number of eyed embryos placed into each RSI. Counts of live fry were minimum numbers of fry produced. Fry survivals were estimated for 124 of the 129 RSIs by year and release site to address objectives 4 and 5.

Data analyses.— We computed bootstrap medians and 95% CIs for green-to-eyed egg survivals and eyed-embryo-to-fry survivals (Agresti and Coull 1998; Carpenter and Bithel 2000; Mangiafico 2016) because survival estimates were not normally distributed (Q–Q plots and Shapiro–Wilk test: $P < 0.001$; Crawley 2007). We used box plots to compare green-to-eyed egg survivals by donor stock and fry survivals by year and RSI location.

To assess factors that might have affected survivals, we used mixed models under an assumption of a binomial distribution incorporating the Gauss–Hermite quadrature approximation to the log-likelihood ("glmer" function in "lme4" package for R; Bates et al. 2015). Our response variables for the two survival analyses consisted of two columns, one for number of successes (eyed eggs or fry) and the other for number of failures (green minus eyed eggs or eyed embryos minus fry). Fixed effects of hatchery versus wild, year, and donor stock were assessed for green-to-eyed egg survivals, and fixed effects of year and RSI site were assessed for fry survivals. Initial analyses indicated that these data were overdispersed, so we added a random-level variable for observation to account for this problem (Bates et al. 2015). Adding the random effect of observation (either egg lot or RSI) in these two analyses improved the adequacy of the models (Markatou and Sofikitou 2019; using Bayesian information criteria [BIC] and the difference in BIC values from the top model [ΔBIC] > 2,000), so we evaluated models that included observation as a random effect.

To evaluate objectives 1, 2, and 3 for green-to-eyed egg survival data we tested models that compared effects of spawning wild versus hatchery adults, donor stock, and year as fixed effects and individual egg lots as a random-level effect. To evaluate objectives 4, 5, and 6 for fry survival data, we included water temperature, year, and RSI site (i.e., location) as fixed effects and individual RSIs as a random-level effect. We used the Nelder–Mead optimization algorithm with 200,000 iterations to fit our data to these mixed models because it is a statistically robust algorithm (Nelder and Mead 1965; Bates et al. 2015; Houllier and Lépine 2019). We tested all model combinations that included the fixed effects using BIC values (Schwarz 1978; Ferguson et al. 2019; Jerde et al. 2019).

If evidence ($\Delta\text{BIC} \geq 4$; Jerde et al. 2019) supported a model with a single fixed effect, we present bootstrapped median estimates and associated 95% CIs for each level of that fixed effect (i.e., year as a fixed effect and each year is a level). Multiple comparisons of medians were conducted using a bootstrap procedure with 2,000 iterations (Agresti and Coull 1998; Chmiel and Gorkiewicz 2012). We used the R statistical package to conduct all statistical tests (version 3.2.3; R Core Team 2015). Statistical significance was set at $P < 0.05$.

Temperature Effects on Emergence Timing

We used Onset Optic Stowaway or Hobo temperature sensors (Onset Computer Corporation, Bourne, Massachusetts) to estimate mean daily water temperatures experienced by embryos from the time they were translocated into each RSI until the last fry was released from the capture bucket. We used thermal units (Trudgill et al. 2005) estimated for Westslope Cutthroat Trout from eye-up to hatch at Montana hatcheries along with field water temperature data to estimate the number of days RSIs would need to be monitored.

RESULTS

Survival Estimates

Green-to-eyed egg survival.— Green-to-eyed egg survival was high (median, 89.2%; 95% CI, 85.9–91.0%), differed between wild and hatchery stocks, and varied among wild stocks (Figure 3; Table 2). Models that contained either the fixed effect of wild versus hatchery stock or individual donor stock explained variation in our data better than other fixed effects based on BIC, supporting objectives 1 and 3, but not the year effect of objective 2 (Table 3). A model evaluating donor stock within year did not converge, probably because the model overfit our data set and was therefore not included. Green-to-eyed egg survivals were significantly different ($P < 0.01$) between progeny from wild-spawned adults (median, 91.0%; 95% CI, 88.7–93.7%) and progeny from hatchery-spawned adults (median, 81.7%; 95% CI, 74.9–88.4%), strongly supporting objective 1. Among donor stocks, embryos from Muskrat Creek parents had the highest survival to the eyed stage (median, 95.1%; 95% CI, 92.9–96.1%), whereas embryos from White's Creek parents had the lowest (median, 64.5%; 95% CI, 54.0–84.2%; Table 2). Median survivals of all donor stocks, except White's Creek, were from 80.0% to 95.1%. Survivals of a few egg lots from Muskrat and Ray Creek parents were lower than 20% (Figure 3). Green-to-eyed egg survivals of the Sun Ranch and Washoe Hatchery stocks were not significantly different ($P = 0.20$; Table 2).

TABLE 2. Mean and bootstrap median green-to-eyed egg survivals by donor stock (W = wild, H = hatchery); *n* denotes the number of egg lots. The results of bootstrap comparison tests are indicated by letters (w, x, y, z), where different letters indicate significantly different medians at $P < 0.05$.

Donor stock and overall total	*n*	Mean (%)	Median (%)	Median 95% CIs
White's Creek (W)	25	62.8	64.5 z	54.0–84.2
Sun Ranch (H)	29	74.2	80.0 zy	69.9–82.4
Washoe Park (H)	42	74.5	86.6 yx	76.6–89.8
Ray Creek (W)	68	79.6	89.0 x	82.4–93.2
Bray's Creek (W)	7	75.0	90.4 zyx	42.3–94.3
Muskrat Creek (W)	73	87.6	95.1 w	92.9–96.1
Overall	244	78.6	89.2	85.9–91.0

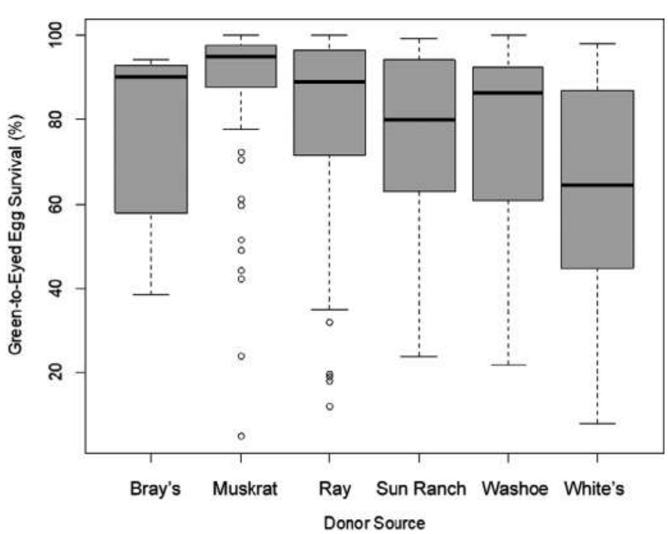

FIGURE 3. Box plots of green-to-eyed egg survivals by donor stock, where the horizontal line in the box is the median, the box dimensions represent the interquartile range (IQR), the whiskers are 1.5 times the IQR in addition to the IQR, and the points are outliers. Donor stocks are Bray's Canyon Creek (Bray's), Muskrat Creek (Muskrat), Ray Creek (Ray), Sun Ranch Hatchery (Sun Ranch), Washoe Park Hatchery (Washoe), and White's Creek (White's).

Fry survivals in RSIs.— Fry survivals from RSIs were relatively high (median, 75.6%; 95% CI, 72.2–79.0%) and varied by year and site, while water temperature appeared to exert a limited influence (Figure 4). The model that included year as a fixed effect was weakly better than the model than included water temperature as a random effect ($\Delta BIC = 3.0$) and much better than the model that included RSI site as a fixed effect ($\Delta BIC = 18.0$; Table 3). However, the coefficient for water temperature was not significant ($P > 0.30$) in the regression model. Fry survivals in RSIs varied by year, supporting objective 4, but there was less evidence that RSI site or temperature were statistically important, objectives 5 and 6.

Median fry survivals from RSIs were highest in 2007 (82.5%; 95% CI, 78.3–84.4%) and lowest in 2010 (62.3%; 95% CI, 59.9–69.4%) and declined from year to year as RSI sites were progressively moved lower in the basin

TABLE 3. Comparison of mixed models, where "Model" shows the model tested ("Wild" is wild versus hatchery; "Lot" is egg lot), "df" is degrees of freedom for each model, "AIC" and "BIC" are Akaike and Bayesian information criteria, "ΔBIC" is the difference in BIC values from the top model, "logLik" is the log-likelihood, and "ΔLogLik" is the difference in log-likelihood values from the top model. Models for each analysis are sorted from lowest to highest BIC scores.

Model	df	AIC	BIC	ΔBIC	logLik	ΔLogLik
Green to eyed eggs						
~ Donor + (1|Lot)	7	2,242.0	2,266.4	0.0	–1,114.0	12.1
~ Wild + (1|Lot)	3	2,258.2	2,268.7	2.2	–1,126.1	0.0
~ Donor + Year + (1|Lot)	10	2,236.5	2,271.4	5.0	–1,108.2	17.9
~ Year + (1|Lot)	5	2,260.4	2,277.9	11.4	–1,125.2	0.9
Eyed embryos to fry in RSIs						
~ Year + (1|RSI)	5	1,236.5	1,250.6	0.0	–613.3	6.3
~ Temperature + (1|RSI)	3	1,245.2	1,253.6	3.0	–619.6	0.0
~ Site + (1|RSI)	9	1,243.3	1,268.6	18.0	–612.6	6.9

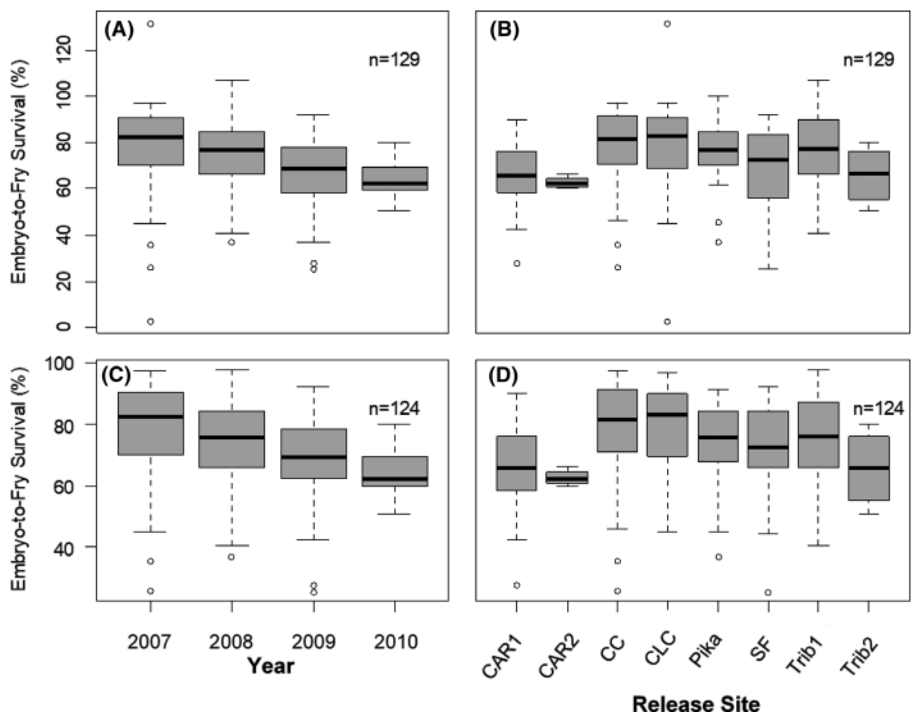

FIGURE 4. Eyed embryo to emergent fry survivals from RSIs by **(A)**, **(C)** year and by **(B)**, **(D)** RSI site. For the box plots, the horizontal line in each box is the median, the box dimensions represent the interquartile range (IQR), the whiskers are 1.5 times the IQR in addition to the IQR, and the points are outliers. The top two graphs (A and B) show the full data set with 129 RSIs, and the bottom two graphs (C and D) show truncated data of 124 RSIs after removing five RSIs that had known problems. Abbreviations for release sites are as follows: CAR1 = upper Carpenter Creek, CAR2 = lower Carpenter Creek, CC = upper Cherry Creek, CLC = upper Cherry Lake Creek, Pika = lower Pika Creek, SF = lower South Fork Cherry Creek, Trib1 = mouth of unnamed tributary below Cherry Lake Creek, and Trib2 = mouth of unnamed tributary above Carpenter Creek.

(Table 4; Figure 1). Fry survivals from RSIs differed among RSI sites, but these differences followed the same pattern as year, with the highest survivals in the two upper sites used in 2007 (Cherry Lake Creek and upper Cherry Creek; Figure 1) and some of the lowest survivals in those sites lower in the basin used in 2010 (lower Carpenter Creek and the lower unnamed tributary; Table 4).

Fry Emergence Dates

Fry left RSIs from 8 to 45 d after the release of eyed eggs into RSIs. Fry began entering capture buckets from 8 to 38 d (mean, 19 d) after eyed embryos were placed into RSIs (Figure 5). The last fry entered capture buckets after 14 to 45 d following embryo translocation (mean, 28 d; Figure 5). The latest emerging fry were captured during

TABLE 4. Mean and bootstrap median eyed-embryo-to-fry survivals in RSIs by year and site; "*n*" denotes the number of RSIs. The results of bootstrap comparison tests are shown by letters (w, x, y, z), where different letters indicate significantly different medians at $P < 0.05$. See Figure 1 for site abbreviations.

Year, site, and overall total	*n*	Embryos released	Fry survival: Mean (%)	Fry survival: Median (%)	Fry survival: Median 95% CIs
Year					
2010	8	1,618	64.2	62.3 z	59.9–69.4
2009	30	8,265	67.1	69.2 zy	65.0–75.7
2008	40	11,732	74.8	75.7 y	72.3–80.6
2007	46	14,123	77.8	82.5 x	78.3–84.4
Site					
CAR2	4	799	62.7	62.3 z	60.1–66.2
CAR1	16	4,241	64.9	65.8 zy	62.2–75.6
Trib2	4	819	65.7	66.1 zyx	50.7–79.9
SF	14	4,024	69.7	72.3 yxw	67.3–82.8
Pika	20	5,777	73.8	75.7 yxw	70.8–82.1
Trib1	20	5,955	75.8	76.0 yxw	71.0–82.1
CC	24	7,166	76.6	81.4 w	74.6–88.0
CLC	22	6,957	75.7	83.1 w	75.4–85.1
Overall	124	35,738	73.4	75.6	72.2–79.0

late August and early September. Based on field water temperatures we measured or predicted during embryo incubation in RSIs (mean, 10°C; range, 6.6–14.5°C) and existing thermal unit estimates for incubating Westslope Cutthroat Trout, we estimated RSIs would need to be monitored from 10 to 40 d.

DISCUSSION

Remote site incubators were an effective method for starting a conservation population of Westslope Cutthroat Trout in more than 30 km of vacant stream habitats over a 4-year period in upper Cherry Creek. The overall survival rate from fertilization of green eggs to fry emergence from RSIs was about 67% (89% green-to-eyed egg survival multiplied by 75% eyed-embryo-to-emergent-fry survival). Remote site incubators produced over 26,500 Westslope Cutthroat Trout fry that entered the upper Cherry Creek basin.

We observed and sampled numerous adult Westslope Cutthroat Trout, spawning adults, and newly emerged fry throughout the basin from 2011 to 2016 (Clancey et al. 2019), providing evidence that fry produced from RSIs grew to maturity and successfully spawned. Postrestoration population density estimates of Westslope Cutthroat Trout 5 to 9 years following RSI introductions in five long-term monitoring sections were equal to or higher than prerestoration density estimates of Rainbow Trout and Brook Trout (Clancey et al. 2019), evidence that this population became well established within 10 years.

Survival Estimates

We found significantly higher green-to-eyed egg survivals in progeny from wild-spawned adults versus hatchery-spawned adults, answering objective 1 and showing that embryos from wild stocks may be used to successfully start a conservation population. We also found significant differences among individual donor stocks, addressing objective 3. Our findings were comparable to findings from a companion laboratory study (Drinan et al. 2012). The differences may have been related to (1) inherent stock differences (Negus 1999; Drinan et al. 2012), (2) slightly different handling of adults and eggs during the spawning, fertilization, and transport process (Wagner et al. 2006), (3) variation in quality of eggs at time of spawning (Smith et al. 1983), or (4) a combination of these factors.

Variability in survivals among egg lots within donor stocks was relatively high. Eight egg lots had exceptionally low green-to-eyed egg survivals (<20%), which might have been related to egg quality (stripping gametes from females when they were not yet fully ripe, when they had already spawned, or whose eggs had begun resorption), nonviable males, or handling differences (Crim and Glebe 1990; DeGaudemar and Beall 1998; Mohagheghi Samarin et al. 2015; Figure 3). Using sperm from at least two males to fertilize each egg lot to ensure all eggs are fertilized is a common hatchery practice (e.g., Davis 1967); however, we used a single male's sperm for each egg lot because we used genetic markers to back-assign progeny to their parental pair for other research questions.

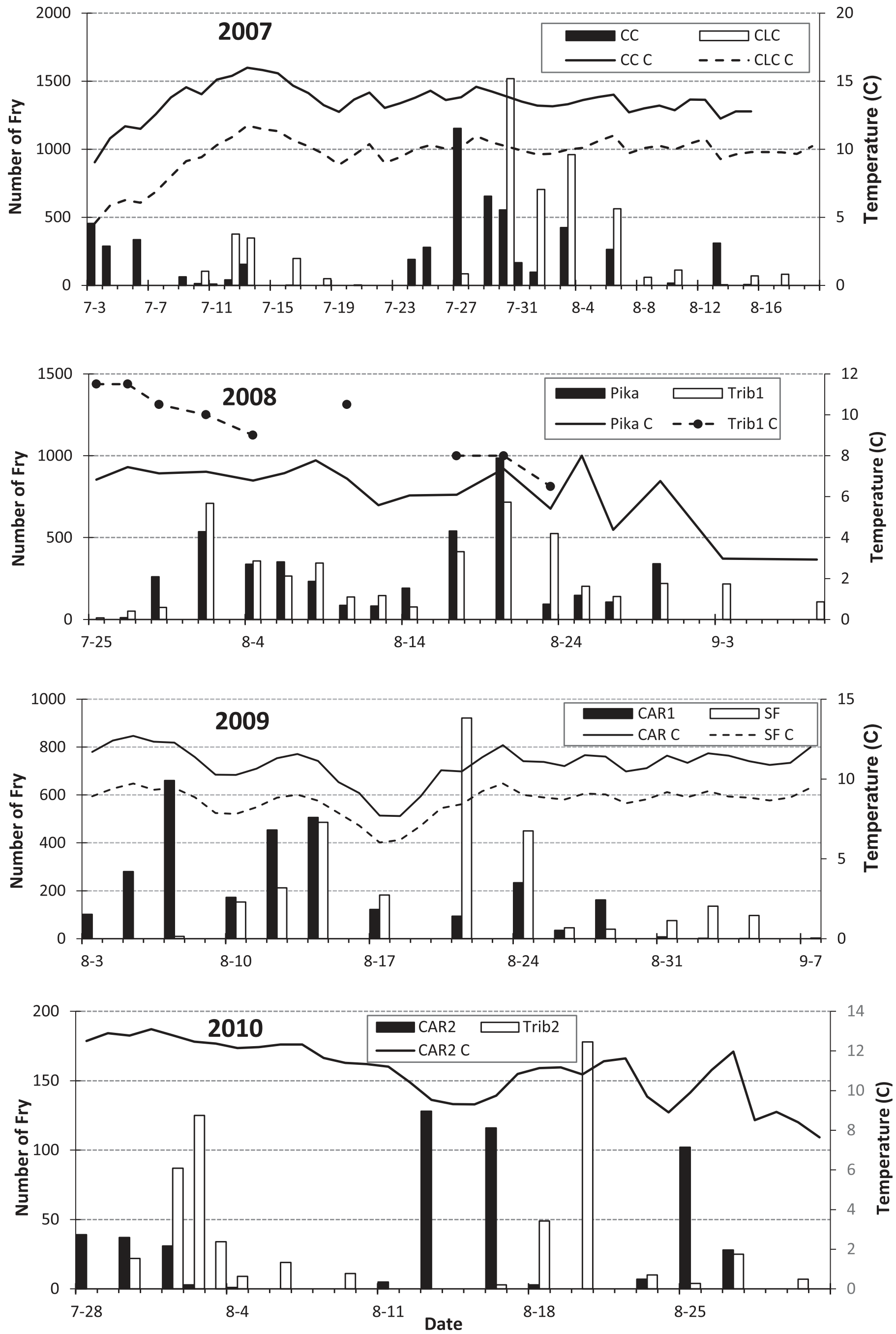

FIGURE 5. Number of fry counted and released from RSI capture buckets by date (bars), and average daily water temperatures (°C) at RSI locations (lines). Each panel shows a different year (2007–2010). Abbreviations for the site codes given in the figure legends are as follows: CAR1 = upper Carpenter Creek, CAR2 = lower Carpenter Creek, CC = upper Cherry Creek, CLC = upper Cherry Lake Creek, Pika = lower Pika Creek, SF = lower South Fork Cherry Creek, Trib1 = mouth of unnamed tributary below Cherry Lake Creek, and Trib2 = mouth of unnamed tributary above Carpenter Creek. The lines (solid and dashed) show average daily water temperature (°C) at each site, indicated by the site abbreviation followed by "C" in the figure legends. For the Trib1 site in 2008, water temperature points show grab samples of water temperatures measured during days RSIs were monitored. No water temperature data were collected at the Trib2 site in 2010.

Survivals of fry from RSIs were high among years and sites (median range, 62–83%), resulting in similar stocking rates of fry throughout the upper Cherry Creek study area. Andrews et al. (2016) subsequently documented that age-1 survivals of fry produced from RSIs in upper Cherry Creek did not differ significantly by RSI introduction site. They found that sampled age-1 survivors represented 77% of all contributing parental pairs and that all six donor stocks had contributed some age-1 individuals. Introduction of embryos from most donor stocks at most sites may have contributed to the consistent success we saw among sites, though we suggest that evaluating site effects more rigorously is an area of future research worth pursuing.

Survivals we documented from the green egg to fry stage were probably higher than they would have been if we had placed recently fertilized embryos into RSIs earlier in the summer prior to eye-up. Minimizing the time embryos spend in RSIs and limiting that time to periods when stream flows are lower reduces risks of both catastrophic and progressive failures of fry production from RSIs (i.e., Donaghy and Verspoor 2000).

Water Temperatures

We saw little evidence that water temperature affected fry survivals but some evidence that water temperature influenced embryo incubation timing. Differences in optimal incubation temperatures existed among different Westslope Cutthroat Trout donor stocks during the green-to-eyed egg phase in the laboratory (Drinan et al. 2012), but all our fertilized green eggs, except for Washoe Park eggs, were incubated to the early eyed stage at temperatures of 8°C to 10°C at Sun Ranch Hatchery. Differences in survival among stocks in the laboratory only became apparent at temperatures of 14°C (Drinan et al. 2012).

Water temperatures in upper Cherry Creek were apparently not cold or warm enough to significantly affect survivals of incubating embryos. The Pika Creek site was the coldest site and the upper Cherry Creek site was the warmest site where RSIs were located (Figure 5). The Pika site had an intermediate median fry survival compared with the other RSI sites (Table 4), with water temperatures from 6°C to 8°C during most of the RSI incubation period (Figure 5). These temperatures were slightly higher than temperatures that led to lower survivals in other studies (Stonecypher et al 1994; Coleman and Fausch 2007). The warm upper Cherry Creek site had water temperatures from 12°C to 15.5°C during the incubation period (Figure 5) and had relatively high fry survivals (Table 4), even though the upper range of observed temperatures surpassed the 14°C found by Drinan et al. (2012) to influence incubation survivals.

Management Implications

Remote site incubators offered a viable alternative for translocating native fish to start a conservation population of Westslope Cutthroat Trout. We translocated small numbers of embryos (mean, 119; SD, 88) from each of a high number of family groups ($n = 258$) from six different donor stocks using RSIs. Incorporating more family groups and potentially more donor stocks in a translocation effort provides higher genetic diversity (Stockwell et al. 1996; McLean et al. 2008; VanDoornik et al. 2011) and better chances of success (Caroffino et al. 2008; Vincenzi et al. 2012). We recommend using numerous egg lots and RSIs per site to reduce the influence of poor survival from a few egg lots or RSIs on restoration success.

Managers should strive to maximize the initial effective population size (N_e) to promote genetic diversity when starting new or supplementing existing native salmonid populations. They should focus their efforts on spawning as many wild adults from as many different source populations as feasible. This strategy will also facilitate preserving the genetic legacy currently represented in extant populations. We acknowledge that using RSIs to translocate embryos is more labor intensive than transferring fry or older-aged fish but suggest that this additional effort may be justified to increase genetic diversity and increase the likelihood of long-term success, reduce risks of transporting pathogens or parasites, and reduce potential population-level impacts on donor populations by removing gametes rather than older individuals.

Eggs can be fertilized and water-hardened in the field in an iodophor solution to disinfect them (i.e., 5 to 50 mL/L; Pravecek and Barnes 2003). We recommend using sperm from at least two males to fertilize each egg lot. These embryos should then be transported to an isolation facility where they can be incubated to the early eyed stage prior to transporting them to RSIs. Incubating these embryos to the early eyed stage in a facility where water temperatures and flows can be controlled allow them to be introduced into RSIs during the early to midsummer period, reducing the length of time embryos need to be incubated in RSIs. This will reduce field maintenance costs and potential failures of RSIs and should increase overall egg-to-fry survivals.

The RSIs need to be maintained every 2 to 5 d to ensure that water flows into the RSIs provide the 30 cm of depth needed to keep embryos alive. Stream flows were dropping daily during RSI deployment in our study area, so frequent maintenance was crucial to our success. Periodic maintenance also allows for quantifying fry production from RSIs.

We successfully started a viable Westslope Cutthroat Trout conservation population in over 30 km of suitable stream habitat within the upper Cherry Creek basin over the course of 4 years using RSI introductions of embryos. This success clearly illustrates that RSIs are a viable method for translocating a genetically diverse group of native salmonids into relatively large areas of connected habitats. It also demonstrates how coordination between conservation managers and researchers allows for better evaluations of native salmonid conservation efforts.

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