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Few Impacts of Introduced Cutthroat Trout (*Oncorhynchus clarki*) on Aquatic Stages of Boreal Toads (*Anaxyrus boreas boreas*)

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ABSTRACT.—**Introduced salmonids can impact aquatic ecosystems through direct predation and indirect effects. We explored the effects of introduced Cutthroat Trout (*Onchoryncus clarki*) on Boreal Toad (*Anaxyrus boreas boreas*) survival and habitat use during two aquatic life stages, the embryo and tadpole, at Boreal Toad breeding sites with and without Cutthroat Trout. We found no difference in embryo survival and higher tadpole survival at the site with Cutthroat Trout. Cutthroat Trout are unlikely to use the shallow areas where Boreal Toad eggs are deposited; however, during the tadpole stage, Cutthroat Trout and tadpoles overlap broadly in near-shore aquatic habitats. Frequency of tadpole habitat use is lower in Cutthroat Trout–used areas, but we observed no behavioral or temporal avoidance of Cutthroat Trout by tadpoles. Our results suggest that Cutthroat Trout do not have a negative effect on Boreal Toad embryo or tadpole survival in wild settings and that Cutthroat Trout presence does not preclude tadpoles from using habitats.**

Human-facilitated introductions of nonnative species pose a significant threat to taxa around the globe (Wilcove et al., 1998; Clavero and Garcia-Berthou, 2005). Classic examples of invasive species involve movements among continents, but nonnative species introduced over shorter distances also have dramatic effects on native communities, such as in high-elevation lakes that are not connected to other water bodies (Rahel, 2007). In the past 2 centuries, humans have intentionally introduced many fishes, especially salmonids, into areas where they were not historically present, including 60% of all high-elevation (>800-m) lakes in the western United States (Bahls, 1992). Introduced salmonids drastically alter trophic interactions in these systems and can lead to population declines or local extinction for amphibian species whose larval stages are vulnerable to fish predation (Pilliod and Peterson, 2001; Knapp, 2005; Pilliod et al., 2010; Amburgey et al., 2014).

To date, most trout–amphibian interaction studies report a reduction in amphibian distribution and abundance (e.g., Bradford et al., 1998; Knapp, 2005; Welsh et al., 2006). Few studies have experimentally investigated the potential direct or indirect mechanisms by which trout impact different amphibian life stages (but see Tyler et al., 1998; Kiesecker et al., 2001; Vredenburg, 2004; Pearson and Goater, 2009; Kenison et al., 2016). Knowledge of the mechanisms by which trout negatively affect amphibian life stages may assist managers tasked with conserving declining populations.

The Boreal Toad (*Anaxyrus boreas boreas*) has declined throughout the southern Rocky Mountains (Carey, 1993; Muths et al., 2003; Mosher et al., 2018) and is listed as an endangered, or tier 1, species in Colorado, Wyoming, and New Mexico. Regional declines are primarily attributed to chytridiomycosis, a disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Muths et al., 2003; Scherer et al., 2005); however, some populations have declined despite low prevalence of the pathogen (Muths and Scherer, 2011). One such population is in Spruce Lake in Rocky Mountain National Park, where a small population of Boreal Toads is declining despite high adult survival (Muths and Scherer, 2011). Eggs are deposited at the site in most years, but recruitment into the adult breeding population is low (Muths and Scherer, 2011), indicating low survival of the aquatic or subadult life stages. One hypothesis for the low recruitment is potential impacts of introduced Greenback Cutthroat Trout (*Oncorhynchus clarki stomias*), a listed subspecies under the Endangered Species Act, on Boreal Toad aquatic life stages. In 1991, Cutthroat Trout, believed to be greenbacks, were introduced to Spruce Lake, which was historically fishless (USFWS, 1998). Bufonid eggs and tadpoles contain bufotoxins, making them unpalatable to many vertebrate predators, including trout (Licht, 1968; Kats et al., 1988; Crossland and Alford, 1998); however, laboratory studies suggest that trout frequently attempted to ingest toad tadpoles, reducing tadpole survival probabilities (Grasso et al., 2010; Lanier et al., 2017). Trout affect tadpole survival in other amphibians by altering trophic interactions (e.g., by reducing the populations of aquatic insects; Knapp et al., 2001) or by reducing the amount of time that tadpoles spend in resource-rich areas (Knapp et al., 2005; Creel and Christianson, 2008).

We explored potential interactions between these two species of concern in a natural setting to assess whether findings from previous lab-based studies (Lanier et al., 2017) hold in the field. We determined the degree of overlap between Cutthroat Trout and Boreal Toad habitat use during two periods corresponding to different aquatic life stages for toads (embryo and tadpole) and estimated Boreal Toad survival through metamorphosis at ponds with and without Cutthroat Trout. We hypothesized that Cutthroat Trout may indirectly impact tadpole survival by altering tadpole habitat use or by attempting to ingest Boreal Toad embryos or tadpoles. If these hypotheses were correct, we would expect to see reduced use or frequency of use by tadpoles of areas of the lake used by Cutthroat Trout as well as lower survival rates for both Boreal Toad life stages in lakes where Cutthroat Trout were present. Our findings will inform recovery efforts for both species within their native ranges.

MATERIALS AND METHODS

Study Area.—Our study was conducted at two Boreal Toad breeding areas in Rocky Mountain National Park: Spruce Lake and a wetland complex near Fay Lakes (Fig. 1). Both high-elevation breeding sites (2,900 and 3,300 m) are surrounded by coniferous forest. Spruce Lake is a permanent lake with a self-

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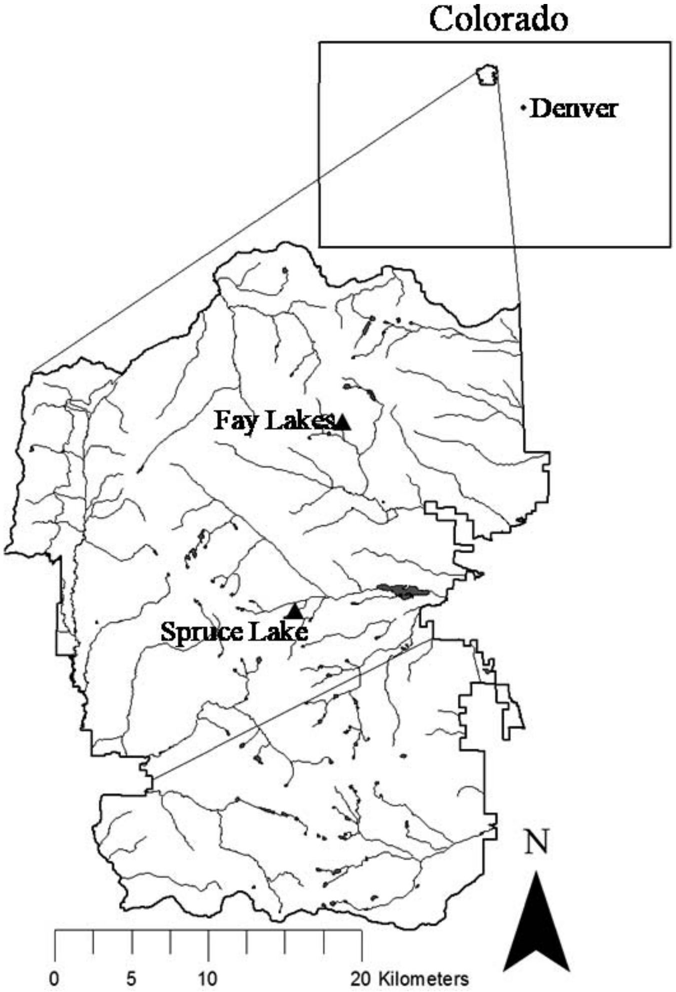

FIG. 1. Map of Rocky Mountain National Park and its major water bodies. The two study sites, Spruce Lake and Fay Lakes, are denoted by black triangles.

sustaining population of greenback Cutthroat Trout (Corn et al., 1997; Muths and Scherer, 2011), and Fay Lakes is a shallow, seasonal, fish-free wetland. All animal care approvals were obtained through Colorado State University's Animal Care and Use Committee.

At Spruce Lake, we explored potential overlap in habitat use between Cutthroat Trout and two early amphibian life stages: embryo and larval (tadpole) stage. Boreal Toads typically breed after ice out in late spring in a sheltered, shallow area on the eastern side of the lake, and tadpoles remain in or near the breeding area until they metamorphose in late summer; Cutthroat Trout and Boreal Toads share aquatic habitat for 2–3 mo (Crockett and Lanier, pers. obs.). We conducted two studies: one study during embryo development and another after tadpoles had hatched and were large enough to be easily seen. Our goal was to determine factors influencing both species' use of aquatic habitat and to test whether Cutthroat Trout presence or intensity of use influence the spatial or temporal distribution of tadpoles.

Embryo Stage Habitat Use: Data Collection.—In 2013 and 2014, we collected Cutthroat Trout detection–nondetection data in areas near Boreal Toad egg mass deposition. We divided the breeding area into four strata of increasing distance from the shore (0–3, 3–6, 6–9, and 9–12 m) and used a stratified random sampling design to estimate Cutthroat Trout use in the area. Four sample plots (2 × 2 m) were randomly chosen each morning and each evening and surveyed the following evening or morning (after a minimum of 8 h), when Cutthroat Trout are active and feeding (Cuenca and de la Higuera, 1994; Sánchez-Vázquez and Tabata, 1998). At each plot, we conducted multiple (2–3) 5-min visual Cutthroat Trout surveys from 4 to 7 m away with at least 15 min between surveys to ensure independence. Cutthroat Trout length was estimated during the survey by comparing the length of a Cutthroat Trout to a ruler placed in each plot. Once all surveys were completed, we collected plot covariates including depth, vegetation density (percentage of the plot with vascular plant matter), temperature, dissolved oxygen (DO), and a qualitative measure of connectivity to the rest of the lake. We measured these same covariates at the egg mass locations and tested for correlation among habitat variables to reduce the number of covariates used in our occupancy analysis.

Embryo Stage Habitat Use: Data Analysis.—We used static occupancy models (MacKenzie et al., 2002, 2018) to estimate Cutthroat Trout habitat use (ψ) and detection probability (p). We expected that Cutthroat Trout habitat use might differ among two size classes: "small" (5–11 cm) and "large" (17–23 cm) trout. We did not detect any 11–17-cm Cutthroat Trout. We hypothesized that the effect of depth and vegetation density on the probability of use would differ between large and small Cutthroat Trout, with large Cutthroat Trout restricted to deeper water, so we modeled interactions between size class and these covariates. We adopted a sequential approach to identify factors influencing Cutthroat Trout habitat use and detection probabilities (see Supplementary Data online) and used program MARK to fit all models (White and Burnham, 1999).

Finally, using estimates from our best supported model and the habitat covariates measured at the egg masses, we predicted the probability of Cutthroat Trout habitat use at the egg masses. Water temperature and DO were measured once in the morning and once in the evening at the egg masses; and because these metrics vary, we averaged the measurements for each period and used these averages to predict Cutthroat Trout use in the morning and evening.

Tadpole Stage Habitat Use: Data Collection.—We explored habitat use and potential interactions between Cutthroat Trout and Boreal Toad tadpoles from 3 to 12 August 2017 in the same area of Spruce Lake. To better differentiate the spatial and temporal features that influence habitat use by both Cutthroat Trout and Boreal Toad tadpoles, we overlaid a grid of 45 plots (2 × 2 m) in the breeding area. Each morning and afternoon during the 10-d season, we randomly selected five plots with replacement, allowing a plot to be visited multiple times during the 10-d season. We navigated to the selected plot and after a 10-min waiting period conducted a 5-min visual survey during which we recorded detection of either species, water temperature, time of day, cloud cover, and wind conditions. Cloud cover and wind conditions were both binary variables, where a 1 indicated clouds that obscured the sun and wind $\geq$10 mph, respectively. We noted any interaction between the two species, such as a change in swimming direction toward or away from the other species, or predation attempts. Once all five plots were surveyed, we returned to the first plot and repeated the process. Successive surveys on the same plot were separated by at least 1 h, and often multiple days. We collected two habitat covariates at each plot, depth, and vegetation density, based on our embryo stage findings (see Results).

TABLE 1. Model selection results for Boreal Toad tadpole habitat use (ψ^{B}) in a two species (greenback Cutthroat Trout and tadpole) occupancy study at a Boreal Toad breeding area in Rocky Mountain National Park. All models had the same structure for Cutthroat Trout detection ($p^{A} = r^{A}$; veg + temp), tadpole detection (p^{B}; varying by Cutthroat Trout use), and Cutthroat Trout use (ψ^{A}; varying by depth). "Trout use" indicates that tadpole use differs in plots predicted to be used by Cutthroat Trout. "." indicates a model in which Boreal Toad tadpole use was constant across all plots. The columns present the model notation, Akaike's Information Criterion adjusted for sample size (AICc), the difference between the model's AICc value and that of the top model (ΔAICc), AICc weights (*w*), number of parameters (*K*), and the deviance of the model.

Trout habitat use model	AICc	ΔAIC_c	*w*	*K*	Deviance
Veg	208.77	0.00	0.60	9	184.96
Veg + trout use	211.83	3.06	0.13	10	184.50
.	212.15	3.38	0.11	8	191.65
Depth + veg	212.80	4.03	0.08	10	185.47
Trout use	214.18	5.41	0.04	9	190.37
Depth	215.55	6.78	0.02	9	191.75
Depth + veg + trout use	215.60	6.83	0.02	11	184.49
Depth + trout use	217.59	8.82	0.01	10	190.26

Tadpole Stage Habitat Use: Data Analysis.—We used the two-species conditional occupancy model (Richmond et al., 2010; MacKenzie et al., 2018) to test whether the presence or detection of Cutthroat Trout altered habitat use or frequency of use by Boreal Toad tadpoles. In this model, the probability that a plot is used by trout during the 10-d season (ψ^{A}) is assumed to be independent of tadpole presence, whereas the probability of tadpole use is conditional on the plot being used or not used by Cutthroat Trout (ψ^{BA} and ψ^{Ba}, respectively). Frequency of use can differ among plots used by a single species or both species. For example, p^{A} and p^{B} denote the detection probabilities of Cutthroat Trout and Boreal Toad tadpoles, respectively, on plots only used by the designated species, whereas r^{A} and r^{B} denote species-specific detection probabilities on plots used by both species. Finally, detection probability of tadpoles at plots used by both species can vary among surveys where Cutthroat Trout were seen (r^{BA}) or not seen (r^{Ba}).

We tested for potential Cutthroat Trout and Boreal Toad tadpole interactions at different spatial and temporal scales. If tadpoles are excluded from plots by Cutthroat Trout, we would expect $\psi^{BA} < \psi^{Ba}$. Alternatively, tadpoles may not completely avoid areas used by Cutthroat Trout, but simply use these areas less frequently, resulting in a lower detection probability of Boreal Toad tadpoles at plots used by Cutthroat Trout (i.e., $r^{B} < p^{B\cdot}$). Finally, if tadpoles temporarily avoid plots when Cutthroat Trout are physically present, we would expect $r^{BA} < r^{Ba}$. We assumed trout frequency of use was independent of tadpole use of a plot ($p^{A} = r^{A}$ in all models).

We evaluated the influence of habitat covariates on probability of use and frequency of use for both species. In addition, we modeled detection probabilities as a function of weather conditions and water temperature. We used a sequential approach to identify factors influencing Cutthroat Trout and Boreal Toad tadpole habitat use and detection probabilities (Table 1; see Supplementary Data online). We used information-theoretic model selection to determine the best supported model (Burnham and Anderson, 2002) and fit all models by using MARK (White and Burnham, 1999).

Embryo Survival: Data Collection.—We explored the potential effect of Cutthroat Trout presence on survival probabilities for aquatic life stages of Boreal Toads in 2013, 2014, and 2017. In 2013–2014, we conducted a manipulative field experiment on wild egg masses at Fay Lakes (Cutthroat Trout–free site, control) and at Spruce Lake (Cutthroat Trout site) to estimate embryo survival and tadpole abundance, followed by metamorph surveys to estimate tadpole survival.

FIG. 2. Photograph of two caged Boreal Toad egg mass halves at Spruce Lake. The paired exposed egg mass halves are not visible, but located directly adjacent to each cage.

Once egg masses were deposited, we divided each mass into two approximately equal halves. Each half was gently placed over a white background and photographed. Eggs were counted from the photographs by using the cell counter function in ImageJ (Rasband, 2019). One randomly selected egg mass half was caged and the other was left exposed. Mesh cages (40 × 40 × 15 cm) were constructed of polyvinyl chloride pipe filled with sand and sealed with aquarium-safe silicone sealant (Fig. 2). We stitched window screen material (18 × 16 mesh, with openings roughly 1.5 mm in width) to the frame so that five sides of the cages were enclosed; the bottom remained open to the substrate. These cages likely excluded most predators of Boreal Toad eggs. The exposed half of the egg mass was not caged and remained accessible to predation or disturbance.

As soon as individuals from an egg mass hatched (Gosner stage 20), we used a small aquarium net (12 × 9.5 cm) to sweep the area occupied by recently hatched tadpoles, being careful to not disturb or disperse undetected tadpoles while maintaining constant effort. We counted the number of captured tadpoles after each sweep and placed them in a separate container. This temporary removal method continued until fewer than 10 individuals were captured in successive net sweeps. Upon completion of the sampling, tadpoles were returned to the original area of the egg mass and cages were removed.

Embryo Survival: Data Analysis.—We used a robust design closed-capture model to estimate embryo survival probability for each egg mass half (Pollock et al., 1990; Kendall and Nichols, 1995). We considered each egg mass half a "population" where individuals were censused during the first primary period by using egg counts from our photographs. The second primary period consisted of the numbers of tadpoles captured during each removal sweep, with each sweep treated as a secondary survey. The detection probability was modeled as constant, and the recapture probability was set to 0 to account for the removal design. Embryo survival represents the probability that an egg survived through hatching, that is, the probability that an individual egg survived to the second primary period. We used MARK to fit this model to the temporary removal data from each egg mass half (White and Burnham, 1999).

To control heterogeneity in survival between egg masses not associated with our treatment, we calculated the proportional difference in survival probability between the caged and exposed halves and used the delta method to calculate associated variance:

$$Prop.\ Diff = \frac{\hat{S}_{caged} - \hat{S}_{exposed}}{\hat{S}_{caged}}. \qquad (1)$$

We used program Contrast (Hines and Sauer, 1989) to compare the proportional survival differences between the Cutthroat Trout and control sites to determine whether the presence of Cutthroat Trout influenced Boreal Toad embryo survival.

Tadpole Survival: Data Collection.—We monitored tadpole development at both sites in all 3 yr (2013, 2014, and 2017). One week after metamorphs (individuals at Gosner stage ≥ 45) were first detected, we performed a three-pass temporary removal sample (Muths et al., 2014). Metamorphs were captured, held in small plastic containers, and counted after each pass. When all passes were complete, we batch marked each individual by using a single visible implant elastomer mark and released them at their capture location. After all tadpoles had metamorphosed (1–2 wk later), we performed a second removal sample, counting the number of previously marked and unmarked individuals on each removal pass. In 2014, all metamorphs had emerged from the Fay Lakes site by the first sample, so only one sample was conducted. In 2017, the Fay Lakes site dried before tadpoles could metamorphose.

Tadpole Survival: Data Analysis.—In 2013–2014, we combined the metamorph removal data and estimated tadpole abundances to generate capture histories for each site and year. Capture histories consisted of three primary periods: initial "release" of hatched tadpoles, followed by the two removal samples. Capture histories contained two states: Boreal Toad tadpoles (T; i.e., the estimated number of tadpoles hatched from our embryo survival study) and metamorphs observed during the second and third periods (i.e., the temporary removal samples of metamorphs). Recaptured metamorphs were randomly distributed among individuals from the marked cohort to avoid bias (Converse et al., 2009). In 2017, embryo survival was not estimated, so initial releases consisted of eggs counted from our photographs.

Because of time differences between primary periods and inconsistency in the stage at release, we analyzed data from each site and year separately using a robust design multistate mark–recapture model (Pollock et al., 1990; Kendall and Nichols, 1995; Muths et al., 2014). This model allows for the estimation of three types of parameters: survival probability, transition probability, and detection probability. Survival probability, S_t^k, is the probability that an individual in state k at time t survives and remains in the study area between time t and time $t+1$. We accounted for unequal time intervals between primary periods, thus resulting estimates are weekly survival estimates. Transition probability, ψ_t^{kl}, is the probability that an individual in state k at time t will be in state l at time $t+1$, given that it survives between the two primary periods. Detection probability, $p_{t,j}^k$, is the probability that an individual will be captured during pass j of sample t, given that it is alive and in state k. We used several constraints to address biological reality and statistical identifiability. Transitioning from metamorph to tadpole is impossible, and tadpoles are not captured in the metamorph removal samples, so $\psi_t{}^{MT}$, $p_{2,j}^T$, and $p_{3,j}^T$ were fixed to 0. Three additional assumptions are required for model identifiability (Muths et al., 2014): we assume that all surviving tadpoles have metamorphosed before the final removal sample ($\psi_2^{TM} = 1$), that weekly tadpole survival probability is constant during the study period ($S_{1\_\mathrm{wk}}^T = S_{2\_\mathrm{wk}}^T$), and that detection probability during the final pass was constrained (i.e., $p_{t,j}^M = p_{t,j-1}^M$).

For each site-by-year combination, we tested four detection structures: constant across all passes; varying by pass, but not by removal sample; varying by removal sample, but not by pass; and varying by both pass and removal sample. Models were fit using MARK (White and Burnham, 1999) and ranked using information-theoretic model selection.

Finally, we combined survival estimates from our best supported models from the Boreal Toad embryo and tadpole analyses to derive an estimate of survival through metamorphosis:

$$S^{EM} = S^E \times \left[S_1^T \left(\psi_1^{TM} + (1 - \psi_1^{TM}) S_2^T \right) \right] \qquad (2)$$

In this equation, differences between S_1^T and S_2^T are solely a function of the time between periods, because weekly survival is constant ($S_{1\_\mathrm{wk}}^T = S_{2\_\mathrm{wk}}^T$). In 2017, embryo survival was not calculated separately and is included in the first survival term S_1^T and S^E is removed from the Equation 2. We used the delta method to calculate associated variances.

Results

Embryo Stage Habitat Use.—We surveyed 85 plots near the Boreal Toad breeding area at Spruce Lake and built 105 occupancy models (see Supplementary Data online). The best supported model ($w = 0.10$; Supplementary Table A1.1) suggested Cutthroat Trout use increased with increasing depth, decreased with increasing vegetation density, and increased with increasing temperature (Fig. 3). Larger Cutthroat Trout were more likely to use the sampled area than smaller Cutthroat Trout (model-averaged estimates at mean covariate values: $\hat{\psi}_{large} = 0.15$, SE = 0.07, $\hat{\psi}_{small} = 0.02$, SE = 0.02). The model-averaged detection probability was $\hat{p} = 0.23$ (SE = 0.06).

Using covariate data collected for three egg masses located at Spruce Lake in 2013–2014, the predicted probability that a Cutthroat Trout would use the habitat near the egg masses during an ~30–45-min season was extremely low (range = 0.00011–0.015; Fig. 3).

Tadpole Stage Habitat Use.—We conducted 116 surveys of 41 plots in near-shore habitat at Spruce Lake. We simultaneously observed Cutthroat Trout and Boreal Toad tadpoles in 15 plots during 20 surveys. We observed no predatory behavior between Cutthroat Trout and tadpoles, although Cutthroat Trout were seen ingesting numerous aquatic insects. We did not observe any change in tadpole or Cutthroat Trout behavior when the two species encountered one another.

Consistent with our findings during the embryo stage, we found that Cutthroat Trout habitat use was influenced by depth and vegetation density (cumulative Akaike's Information Criterion adjusted for sample size [AICc] weight, $w_+ > 0.99$ for both covariates; Supplementary Table A2.4), with comparable relationships to those shown in Figure 3. The probability that Cutthroat Trout used a plot at least once during the 10-d season was high; estimated at 0.74 (SE = 0.12), by using an

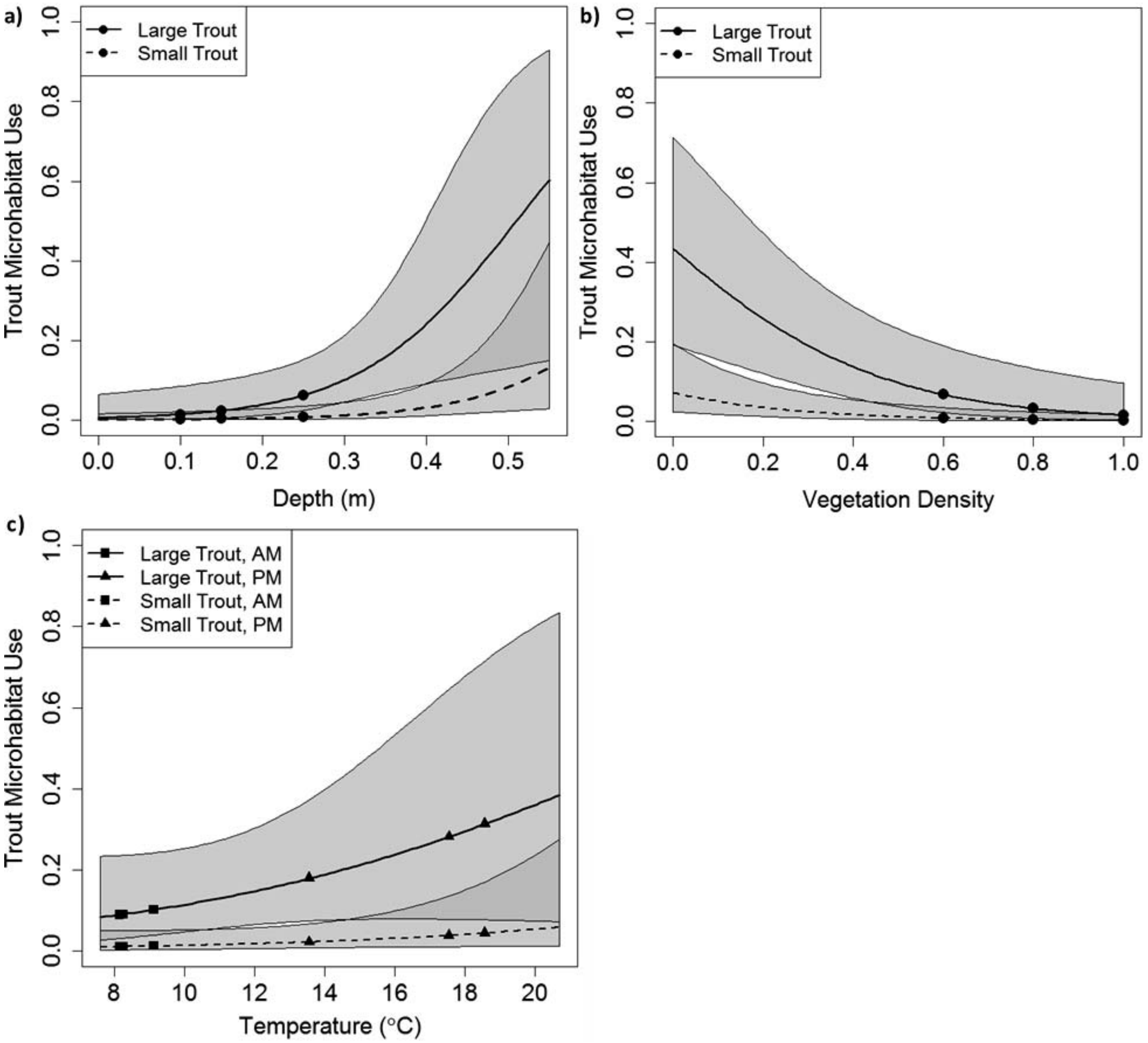

FIG. 3. Estimated relationships between Cutthroat Trout habitat use and water depth (a), vegetation density (b), and temperature (c) by using the most parsimonious occupancy model, ψ(size + depth + veg + temp), p(.), during the embryo stage of Boreal Toad development. Relationships with each covariate is plotted using the average values of other covariates. The dashed line designates the relationship for small Cutthroat Trout, and the solid line represents the relationships for large Cutthroat Trout. Shaded areas represent 95% confidence intervals. In (a) and (b), the circles indicate the vegetation density and predicted use at the three egg mass locations. In (c), the squares indicate the average morning temperatures and predicted use at the three egg mass locations. The triangles represent the same for the average evening temperatures.

intercept-only model. Cutthroat Trout frequency of use increased with temperature and decreased with vegetation density ($w_+ > 0.90$ for both covariates; Supplementary Table A2.1). Cutthroat Trout detection probability estimates ranged from 0.004 (SE = 0.005) for surveys conducted at plots with high vegetation density during cold temperatures to 0.90 (SE = 0.06) for surveys conducted at plots with no vegetation during warm temperatures.

Boreal Toad tadpole habitat use was also influenced by vegetation density (Table 1); tadpoles used nearly all plots with low vegetation density, but tadpole probability of use declined in plots with vegetation densities greater than 0.50. Few plots had vegetation densities higher than 0.50, and average tadpole use probability across all plots was 0.98 (SE = 0.08). Importantly, we found no evidence that Cutthroat Trout influenced Boreal Toad tadpole habitat use; whereas the second-best-supported model does include a Cutthroat Trout effect (Table 1), the inclusion of this effect does not improve model fit. Tadpole frequency of use was lower at plots used by Cutthroat Trout ($r^B = 0.71$, SE = 0.05) relative to plots not used by Cutthroat Trout ($p^B = 0.97$, SE = 0.03), but tadpoles did not avoid plots when Cutthroat Trout were seen during the same survey (Supplementary Table A2.3).

Embryo Survival.—We discovered 10 egg masses from the two sites in 2013–2014 (Table 2), and eggs hatched 5–9 d after discovery (Gosner stage 25). Embryo survival for both caged and

TABLE 2. Estimates of Boreal Toad embryo survival probability ($\hat{S}^E$) and SEs (in parentheses) for egg mass halves that were caged or exposed to predation. There were 10 egg masses found at the control and Cutthroat Trout sites in Rocky Mountain National Park in 2013 and 2014. The proportional difference, defined as the difference in survival probability between the caged and exposed halves divided by the caged survival probability, and associated variance are also given.

Site and year	Egg mass	Caged	Exposed	Proportional difference	Proportional difference variance
Control site (Fay Lakes)					
2013	1	0.01 (0.002)	0.02 (0.002)	−0.47	0.083
	2	0.12 (0.005)	0.14 (0.007)	−0.17	0.0052
	3	0.08 (0.016)	0.04 (0.004)	0.44	0.016
	4	0.05 (0.004)	0.02 (0.002)	0.58	0.0030
	5	0.08 (0.006)	0.01 (0.002)	0.86	0.00072
2014	6	0.40 (0.006)	0.16 (0.004)	0.61	0.00010
Trout site (Spruce Lake)					
2013	7	0.34 (0.012)	0.29 (0.012)	0.13	0.0020
2014	8	0.38 (0.017)	0.13 (0.011)	0.66	0.0012
	9	0.72 (0.038)	0.52 (0.036)	0.28	0.0039
	10	0.32 (0.008)	0.17 (0.006)	0.47	0.00054

exposed egg mass halves was much higher at the Cutthroat Trout site (Spruce Lake survival range = 0.13–0.72) than the control site (Fay Lake survival range = 0.01–0.40; Table 2). This was primarily because of an outbreak of water mold at the control site in 2013, resulting in very low survival. Survival probabilities observed for the control site during 2014 were within the range of those found at the Cutthroat Trout site (Table 2). We found no difference in proportional survival between the Cutthroat Trout and the control site (df = 1, $P = 0.19$); exposed eggs at the Cutthroat Trout site did not show greater decreases in Boreal Toad embryo survival than exposed eggs at the control site.

Tadpole Survival.—Despite egg deposition and variable embryo survival, Boreal Toad metamorphs were only observed at the Cutthroat Trout site in 2013 and at the control site in 2014 (Table 3). Similarly, egg masses were deposited at both sites in 2017 (7,992 and 2,371 eggs at the Cutthroat Trout and control site, respectively), but metamorphs were only observed at the Cutthroat Trout site (Table 3).

Boreal Toad metamorph detection probability varied among pass, removal sample, and year, ranging from 0.20 to 0.68. There was no consistent pattern to which sample or pass had higher detection probabilities. Tadpole survival varied across sites and years, ranging from 0.00 to 0.66 (Table 3). In years when metamorphs were detected, survival to metamorphosis was higher at the Cutthroat Trout site than the control site (Table 3), but never exceeded ~0.20. In addition, although there was only one year (2014) with complete reproductive failure at the Cutthroat Trout site, reproductive failure occurred in two of the 3 yr at the control site (2013 and 2017).

DISCUSSION

We found no evidence that Cutthroat Trout negatively affect aquatic stages of Boreal Toads, despite the two species sharing habitats during the Boreal Toad tadpole stage. This finding agrees with numerous observational studies that show no negative impacts of trout on toad distributions (e.g., Corn et al., 1997; Bull and Marx, 2002; Knapp, 2005; Welsh et al., 2006), but differs from lab experiments involving toads and trout (Grasso et al., 2010, Lanier et al., 2017). Those experimental studies suggest that naïve trout attempt to eat toad tadpoles before rejecting them (Grasso et al., 2010) and that these attempts reduce tadpole survival probabilities (Lanier et al., 2017). Lanier et al. (2017) found no evidence of trout learning to avoid toad tadpoles, but other studies have demonstrated a capacity for predatory fish to learn to avoid unpalatable prey (e.g., Kruse and Stone, 1984; Nelson et al., 2010, 2011; Szuroczki and Richardson, 2011). Boreal Toads are the only amphibian species found at Spruce Lake, which may allow the Cutthroat Trout to easily distinguish them as unpalatable. Moreover, if predatory behavior existed in natural environments, we would expect Boreal Toad tadpoles would alter their behavior and avoid areas frequented by Cutthroat Trout. We found no evidence of avoidance in our study, corroborating similar findings in laboratory studies (e.g., Kiesecker et al., 1996), suggesting that unpalatability is a sufficient protection for Boreal Toads against Cutthroat Trout predation.

We found that Cutthroat Trout generally avoid the very shallow areas (usually ≤15 cm) where Boreal Toads deposit their egg masses (Holland et al., 2006; Fig. 3). Moreover, we found no difference in survival rates between exposed and caged egg masses at the Cutthroat Trout site. Collectively, these findings suggest few or no interactions between Cutthroat Trout and Boreal Toads during embryo development. Clutch sizes at both sites in this study were comparable to clutch sizes seen in other studies (e.g., Maxell et al. 2002; Carey et al., 2005).

Boreal Toad tadpoles and Cutthroat Trout overlapped in the habitat they used, but Cutthroat Trout were restricted to areas of deeper water, whereas tadpoles used the entire study area. Tadpole frequency of use was lower in the Cutthroat Trout–used areas, but there was no indication that this difference was because of negative interactions. At a finer temporal scale (during a given 5-min survey), we found no evidence that Boreal Toad tadpoles avoid areas that are simultaneously occupied by a Cutthroat Trout. This is consistent with laboratory studies suggesting that toads do not show behavioral avoidance of fish (Kats et al., 1988; Kiesecker et al., 1996). In

TABLE 3. Estimated survival probability for Boreal Toad embryos ($\hat{S}^E$), tadpoles ($\hat{S}^T$), and from embryo through metamorphosis ($\hat{S}^{EM}$) with associated SEs (in parentheses). $\hat{S}^T$ is tadpole survival through metamorphosis, given by the bracketed part of Equation 2. Tadpoles were documented, but no metamorphs found in three of the six site–year combinations, suggesting complete reproductive failure. Embryo and tadpole survival were not estimated separately in 2017, so we simply report our estimate of $\hat{S}^{EM}$.

Site	Yr	Total eggs deposited	$\hat{S}^E$	$\hat{S}^T$	$\hat{S}^{EM}$
Control site (Fay Lakes)	2013	33,876	0.06 (0.002)	0.00	0.00
	2014	18,065	0.28 (0.004)	0.21 (0.048)	0.06 (0.013)
	2017	2,371		0.00	0.00
Trout site (Spruce Lake)	2013	5,513	0.31 (0.008)	0.66 (0.106)	0.20 (0.033)
	2014	17,097	0.32 (0.007)	0.00	0.00
	2017	7,992		NA	0.17 (0.005)

[a] NA = not applicable.

addition, Boreal Toad tadpole survival probability was higher at the Cutthroat Trout site relative to our control site, although there was a large amount of variation among years at both sites.

Several hypotheses could explain higher embryo and tadpole survival at our Cutthroat Trout site, but we are unable to distinguish between them in this study. Cutthroat Trout alter trophic interactions and could reduce the abundance of predatory aquatic insects (e.g., Knapp et al., 2001), which prey on Boreal Toad tadpoles (Peterson and Blaustein, 1992; Kiesecker et al., 1996). Alternatively, there could be differences in habitat quality unrelated to Cutthroat Trout presence. The control site is an ephemeral pond that is smaller than the Cutthroat Trout site. Water bodies with larger perimeters are associated with toad presence (Welsh et al., 2006) and strictly ephemeral sites make larval toads more vulnerable to desiccation, as was observed at our control site in two of the 3 yr in our study (Table 3). In addition, the pathogen *Batrachochytrium dendrobatidis* is present at the control site, but not at the Cutthroat Trout site; although the pathogen primarily affects adult amphibians, it can also impact the survival probabilities of tadpoles (Garner et al., 2009; Crockett et al., 2020). In addition to these differences in habitat quality, there may be genetic differences between the populations of Boreal Toads at these sites (Samollow, 1980).

Introduced salmonids have drastically altered trophic interactions and limited amphibian distributions in high-elevation lakes in the western United States (e.g., Dunham et al., 2004). Bufonids are thought to be immune to this stressor, but laboratory studies suggest naïve, hatchery-reared trout could lower survival and time to metamorphosis for resident toad populations immediately after release (Lanier et al., 2017). Our study suggests that this negative effect does not persist in native Boreal Toad systems. Boreal Toads use a variety of ephemeral and permanent wetlands for reproduction (Holland et al., 2006; Dodd, 2013), but currently persist in high-elevation sites within their historic distribution in the southern Rocky Mountains (Mosher et al., 2018, Crockett, 2019). These same wetlands and lakes are also targeted for restoration efforts for the threatened greenback Cutthroat Trout, because barriers can be placed downstream to avoid colonization by nonnative salmonids. Our findings suggest that Boreal Toads and introduced Cutthroat Trout can coexist and that these habitats may serve as important refugia for multiple species of concern.

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