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Journal of Herpetology

Journal of Herpetology, Vol. 43, No. 1, pp. 132–138, 2009

Short-Term Effect of Cattle Exclosures on Columbia Spotted Frog (*Rana luteiventris*) Populations and Habitat in Northeastern Oregon

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Abstract.—**Livestock grazing is a common land use across the western United States, but concerns have been raised regarding its potential to affect amphibian populations. We studied the short-term effects of full and partial livestock grazing exclosures on *Rana luteiventris* (Columbia Spotted Frog) populations using a controlled manipulative field experiment with pre- and posttreatment data (2002–2006). Despite a significant increase in vegetation height within grazing exclosures, we did not find treatment effects for egg mass counts, larval survival, or size at metamorphosis 1–2 years following grazing exclosure installation. Water samples taken in late summer showed concentrations of nitrite, nitrate, ammonia, and orthophosphate that were low or near detection limits across all ponds and years. The results of this experiment do not support a hypothesis that limiting cattle access to breeding ponds will help conserve *R. luteiventris* populations in our study area. Further research is needed to evaluate regional variation and long-term effects of grazing exclosures on *R. luteiventris* populations.**

Livestock grazing is one of the most widespread and intensive uses of semiarid landscapes in the western United States (Crumpacker, 1984; Belsky et al., 1999). Small ponds are often created to provide water for livestock, and these ponds can be habitat for amphibians (e.g., Baker and Halliday, 1999; Monello and Wright, 1999; Knutson et al., 2004). Although constructed ponds add to the limited aquatic habitat in arid landscapes, concerns have been raised regarding potential negative effects of cattle on resident amphibians. Several studies in agricultural landscapes have found negative associations between grazing and amphibian species or communities (Jansen and Healey, 2003; Knutson et al., 2004). Mechanisms proposed for negative effects include direct trampling of amphibians (all life stages), water quality degradation associated with livestock waste, and changes to vegetation and soil structure (Boyer and Grue, 1995; Ross et al., 1999; Jansen and Healey, 2003). Changes to vegetation and soils in particular could have positive or negative effects on oviposition options, adult feeding opportunities, and predator-prey interactions. In the absence of grazing, dense vegetation may exclude frogs from preferred oviposition or feeding sites. Despite the ubiquity of grazing in the range of several western amphibian species experiencing population declines, no experimental studies have investigated the management of livestock as it pertains to pond-breeding anurans.

Rana luteiventris (Columbia Spotted Frog) is one of several amphibian species that have declined in parts of their range in the western United States (Corn, 2000; Reaser, 2000; Wente et al., 2005). It is considered a Sensitive Species in Oregon and is a candidate for federal protection under the Endangered Species Act (USFWS, 1997). A variety of threats to the persistence of *R. luteiventris* populations have been identified, including wetland loss, introduced predators, mining, grazing, development, and diseases (USFWS, 1997; Monello and Wright, 1999; Reaser and Pilliod, 2005). *Rana luteiventris* is a species that is highly aquatic seasonally and requires permanent and semipermanent wetlands that have aquatic vegetation and some deeper or flowing water for overwintering (Bull and Marx, 2002; Pilliod et al., 2002). Little information currently exists to assess livestock grazing as a possible threat or to manage grazing in a manner beneficial to this species.

Our study area was in the Blue Mountains of northeastern Oregon in the Wallowa-Whitman National Forest (Fig. 1). Lower elevations in the study area are predominately sage brush (*Artemisia tridentata*) and open forest of Ponderosa pine (*Pinus ponderosa*). Upper elevations and north slopes consist of closed forest of lodgepole

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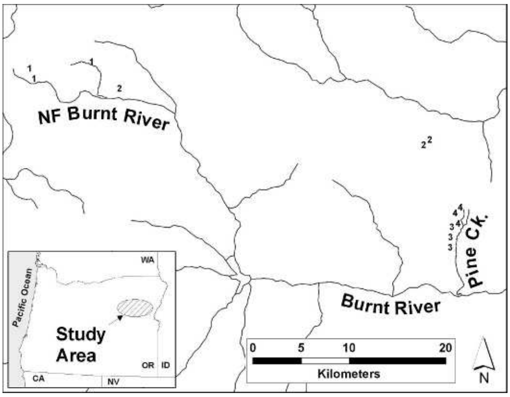

FIG. 1. Map showing location of ponds used to study the effects of grazing exclosures on *Rana luteiventris* populations in northeastern Oregon (2002–2006). Numbers designate block membership.

pine (*Pinus contorta*) and grand fir (*Abies grandis*). Riparian habitats are typically combinations of willow (*Salix* spp.) and seasonally saturated meadows of sedges (*Carex* spp.) and grasses (Poaceae). Precipitation occurs primarily as snow between November and March (Franklin and Dyrness, 1988). The predominant land uses in the vicinity of the study ponds are forestry, mining, and livestock grazing.

In our study area, livestock grazing is widespread and *R. luteiventris* frequently breeds in ponds used by cattle. This indicates an ability of *R. luteiventris* to coexist with cattle grazing in some situations. Our research question is whether limiting the access of cattle to these ponds might benefit *R. luteiventris* populations in this region by increasing egg mass numbers or larval survival. Given declines in this species, it is important that we examine all conservation options. We used a manipulative experiment with pre- and posttreatment sampling to examine the effects of grazing exclosures on *R. luteiventris* and their habitat. We compared *R. luteiventris* egg mass counts, larval survival, size at metamorphosis, water chemistry, and shoreline vegetation among ponds with full, partial, or no exclosure (control) treatments.

MATERIALS AND METHODS

Site Selection.—We initially identified a set of 19 candidate ponds that were suitable for this study. Only apparently permanent, fishless ponds on USFS land, subjected to annual access by cattle and with known breeding populations of *R. luteiventris* were included in this initial pool. From these 19 ponds, we randomly chose 12 ponds to use in the experiment (Table 1). Cattle were stocked at the normal rate prescribed by the U.S. Forest Service, ranging from 24.7–31.2 ha/cow-calf pair in the grazing allotments we used (R. Estes, USFS, pers. comm.). To reduce spatial variation in environmental factors and grazing pressure, we assigned ponds to blocks within an allotment wherever possible (Fig. 1). However, we lost one pond from Block 2 after study initiation and had to replace it with

TABLE 1. Characteristics of ponds in northeastern Oregon where the effects of grazing exclosures on *Rana luteiventris* were studied from 2002–2006.

Pond	Block	Exclosure treatment	Exclosure construction	Area (m^2)	Max depth (m)	Elevation (m)	Number of years of data included in analysis (pretreatment, posttreatment): Egg mass counts	Larval survival	SVL	Vegetation	Water quality
Slab Creek	1	None	–	240	1–2	1,579	3,2	2,2	2,2	2,3	2,3
Winterville	1	Partial	Fall 2003	120	< 1	1,471	2,2	1,2	1,2	1,3	1,3
Little Greenhorn	1	Full	Spring 2005[2]	400	1–2	1,585	4,1	3,1	3,1	3,2	3,2
Black Mountain, Lower	2	None	–	4,200	< 1	1,554	3,2	1,2	2,2	2,3	2,3
Black Mountain, Little	2	Partial	Fall 2003	300	1–2	1,494	3,2	2,2	2,0	2,3	2,1
North Fork Burnt River[1]	2	Full	Spring 2004[2]	360	1–2	1,295	3,2	2,2	2,2	2,3	2,3
Pine Creek E	3	None	–	625	> 2	1,372	3,2	2,2	2,2	2,3	2,3
Pine Creek A	3	Partial	Fall 2003	1,200	> 2	1,353	3,2	2,1	2,1	2,3	2,3
Pine Creek D	3	Full	Spring 2004[2]	1,250	> 2	1,369	3,2	2,2	2,2	2,3	2,3
Pine Creek J	4	None	–	112	< 1	1,539	3,2	–	–	1,3	1,3
Pine Creek I	4	Partial	Fall 2003	120	1–2	1,609	3,2	–	–	2,3	2,3
Pine Creek G	4	Full	Fall 2003	120	1–2	1,579	3,2	–	–	1,3	2,3

[1] This pond replaced the original pond in this block. It is located in an adjacent grazing allotment with higher grazing pressure than the other two ponds. This difference may be somewhat negated by lower cattle access to this pond compared to the other two.
[2] Fence built after breeding, prior to frog recruitment and the arrival of cattle on grazing allotment.

a pond from an adjacent grazing allotment. Distances between ponds within Blocks 1–4 averaged 4.3, 21.8, 0.8, and 0.7 km, respectively, with the high number for Block 2 attributable to the replacement of the full exclosure pond. The replacement pond had parallel data and, thus, only differs from the original pond in that it does not conform to the original blocking scheme. One of each treatment was randomly assigned to the three ponds within each block.

As is typical in this region, most ponds were in riparian areas and were created as part of small mining operations by excavation or impounding. None of our study ponds was being used by miners during the study. Bottom substrates in ponds ranged from cobble to organic muck, and all ponds had areas of submergent or emergent vegetation (mainly *Eleocharis*, *Glyceria*, *Lemna*, *Ranunculus*, *Utricularia*, and algae). Ponds were supplied by a combination of ground water and stream/overland flow. Cattle had access to water sources not involved in the experiment (streams or other ponds) in all four blocks; thus, we consider it unlikely that our exclosed ponds appreciably increased cattle use of the control pond within a block. Such behavior would tend to accentuate any treatment effect.

Grazing Exclosures.—Grazing exclosures were constructed of wooden posts or barbed wire stretched between metal fence posts. We left 1–5 m of riparian vegetation as a buffer between each fence and its pond at high water. Full exclosures completely encircled the pond and entirely excluded cattle from study ponds. Partial exclosures included a fence running across the pond and, thus, completely excluded cattle from a portion of the pond but left at least half open to access by cattle. Fences were approximately 1.5 m in height. Pretreatment data were used to identify the portion of each pond that was most commonly used for oviposition (Pearl et al., 2007). This portion was subsequently fenced in the partial exclosure treatment. Control ponds were not fenced and, thus, allowed full access by grazing cattle.

Exclosures were constructed and installed by the Forest Service between Fall 2003 and Spring 2005 (Table 1). Breeding by *R. luteiventris* occurred in April or May with larval development continuing through metamorphosis in August or September. Cattle had access to the study ponds from late June to September. Because cattle did not access the ponds until after breeding, we considered the first year with fences present to be a transition year. For the egg mass analysis, we included the transition year with pretreatment years because oviposition occurred prior to the time that cattle were first affected by the fences. For larval survival and size at metamorphosis, we omitted the transition year from the analysis because part of the larval period occurred during the pretreatment phase and part during the posttreatment phase. For vegetation and water quality data, we included the transition year data with the posttreatment data because these data were collected at least one month after the cattle first encountered the fences.

Data Collection.—We initiated pretreatment data collection in April 2002. Response variables were monitored for 2–4 years prior to grazing exclosure installation and 1–3 years after treatment depending on the response variable and the timing of fence installation (Table 1). We conducted egg mass counts each spring (late April to early June). Egg mass counts involved crews of 2–3 people searching all areas of ponds <1 m in depth. We considered these counts to be a complete census because of the simplicity of the ponds and the thoroughness of the surveys. Each site received a follow-up survey approximately every week until surveyors were confident that annual oviposition had been completed. Despite repeated surveys for egg masses each year, we never observed egg masses that were stranded by receding water levels. Data from 2006 are the last to be included in the current analysis, but we continue to monitor egg mass counts for future analysis.

Using toe clips as our mark-recapture technique, we estimated the number of metamorphic *R. luteiventris* (Stage 45–46 per Gosner, 1960) at each study pond. We examined a subset of ponds weekly beginning in mid-July to determine the timing of metamorphosis and initiated marking when our visual and dipnetting inspections indicated that $>90\%$ of the larvae had completed transformation. Crews of 2–4 surveyors hand captured juvenile frogs over three consecutive days at each pond. We batch marked all captured frogs with a single toe-clip code that was unique for each day. Toe clips never included interior ''thumbs.'' We measured mass (g) and snout–vent length (mm) of a subset of these captures (usually the first 10–20 individuals captured). At the conclusion of each day's sampling, we released them around the pond to allow them to mix with unmarked frogs. We calculated population estimates with program CAPTURE within program MARK (version 4.3; G. C. White, K. P. Burnham, D. L. Otis, and D. R. Anderson. Utah State University Press, Logan, 1978; White et al., 1982). Based on our survey methods, we elected to use the m(th)-Chao model to generate population estimates. This model incorporates differences among days and among individuals in the probability of being captured. In two cases, only a single metamorph was found; thus, we used this number as the estimate of abundance. We calculated an index of survival from egg mass through metamorphosis (larval survival index) for each pond each year by dividing our estimate of the number of metamorphs by the number of egg masses found that spring. Because one pond in the fourth block had to be replaced (because of access issues) with a pond that did not have estimates of metamorphs, we did not include the fourth block in the analysis of larval survival.

We gauged livestock use intensity at each study pond by monitoring shoreline vegetation in August and September following the livestock grazing season. We established eight square sampling plots (2×2 m) at regular compass bearings from the center of each study pond (0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315°). Each plot was centered on the given compass bearing and positioned with one 2-m side on the water-shore interface. We recorded the maximum height of herbaceous vegetation at six sampling points along the perimeter of each plot (one point in each of the four corners and one point halfway between shoreline and distal edges). Surveyors noted any sampling points that were dominated by rock and, therefore, unsuitable for plant growth. Such points were eliminated from the calculation of mean vegetation height on each plot.

We assessed pond water quality by collecting samples from all study ponds each year using a standardized sampling protocol adapted for pond environments (Wilde and Radtke, 2005). We typically collected water samples twice each year (usually during July and September) although sometimes we collected one or three samples and we failed to collect any samples at one pond in one year. During each water sampling event, we created a composite sample by collecting water at ≥3 points spaced equally around the site. Water was collected by hand-dipping clean 1-liter polyethylene sampling bottles. Collection points were ≥25 cm deep and as far from shore as possible. We avoided inflows and areas of dense aquatic or overhanging vegetation when collecting water samples and avoided entraining surface film and suspended sediments by immersing the sample bottle below the water surface before opening. The composite water samples were kept in a cooler with ice and, within a week, were sent for analysis at the USGS National Water Quality Laboratory in Denver, Colorado. Samples were analyzed for pH, specific conductance, and acid neutralizing capacity according to Fishman and Friedman (1989) and analyzed for nutrients (nitrates, nitrites, ammonia, and orthophosphates) according to Fishman (1993).

Analysis.—We used analysis of variance (S-Plus 6.2) to test whether changes in egg mass counts, survival, growth, and habitat differed among exclosure treatments. The response variable was always the mean of the posttreatment years minus the mean of the pretreatment years at each pond. Models followed a randomized complete block design with variables coding for block (1–4) and treatment (none, partial, or full exclosure). Means are given ±1 SE.

We used a two-tailed paired *t*-test to determine whether the exclosures affected the change in vegetation height. The data were the difference between pre- and posttreatment vegetation height. The test compared changes inside the exclosures (including all vegetation plots from the full exclosure treatment and the enclosed plots from the partial exclosure treatment) to changes outside the exclosures (including all plots from the control ponds and the unenclosed plots from the partial exclosure treatment). Full exclosure ponds were paired with control ponds within blocks and the enclosed portion of partial exclosure ponds was paired with the unenclosed portion. This resulted in eight differences (two from each block) for the analysis.

There were two ponds in our study that each had one year with no breeding. Only one of these ponds was part of the survival analysis, and we treated this as a missing observation for that year because no tadpoles had the opportunity to survive. All other ponds were used for breeding by *R. luteiventris* every year. One other anomaly in the data occurred when we did not find any metamorphs at a pond that dried over the summer before metamorphosis began. We treated this case as zero survival.

Results

Average vegetation height at study ponds ranged from 1.3–24.9 cm pretreatment and from 2.7–36.1 cm posttreatment (Fig. 2A). The exclosures had a significant effect on the change in vegetation height ($t_7 = 3.94$, $P = 0.006$). The average change (post- minus pre-) was 21.0 ± 2.9 cm for plots located inside exclosures and was 3.3 ± 1.7 cm for plots located outside exclosures.

Egg mass counts ranged from 0–32 pretreatment and from 0–19 posttreatment. A trend toward fewer egg masses in the control and partial exclosure ponds posttreatment appeared to be negated in the full exclosure ponds (Fig. 2B), but this pattern was not significant ($F_{2,6} = 0.98$; $P = 0.429$). The larval survival index ranged from 0.33–155 pretreatment and from 0.0–195 posttreatment (Fig. 2C), but we

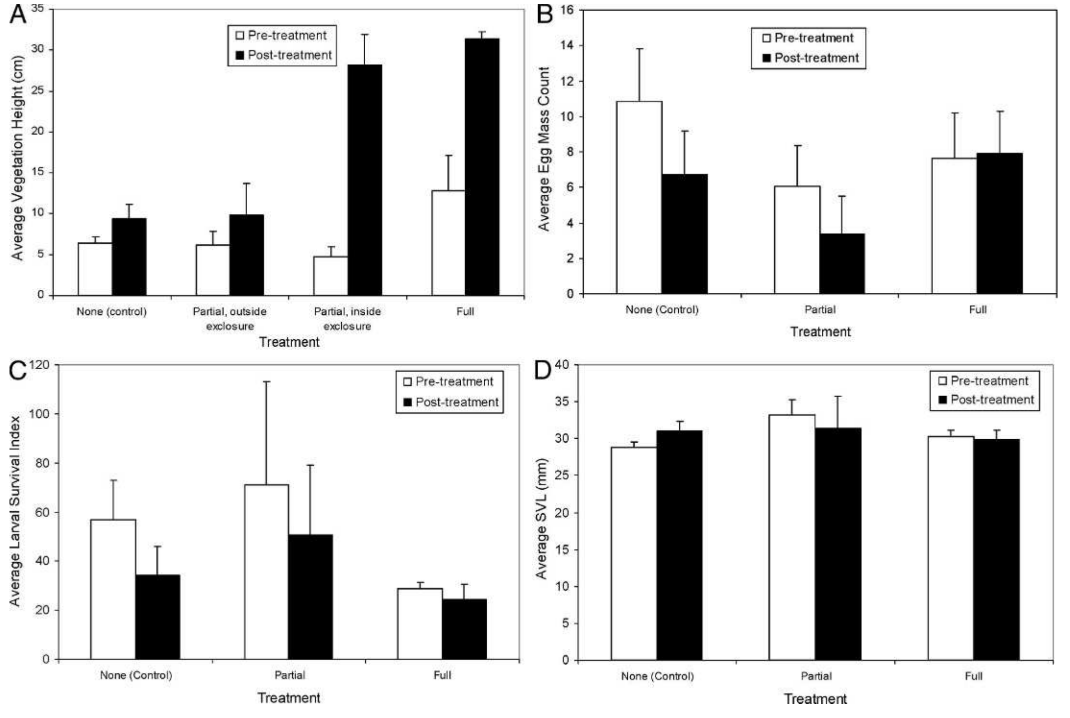

FIG. 2. Responses of *Rana luteiventris* and habitat to grazing exclosures in northeastern Oregon (2002–2006): (A) height of shoreline vegetation; (B) egg mass counts; (C) larval survival; and (D) size at metamorphosis.

found no evidence that changes in larval survival were related to the treatments ($F_{2,4}$ = 0.169; P = 0.850). The mean snout–vent length of metamorphs and juveniles ranged from 24.4–36.7 mm pretreatment and from 22.9–35.7 mm posttreatment (Fig. 2D). The change in size from pre- to posttreatment years was not significantly different among treatments ($F_{2,3}$ = 1.15; P = 0.427).

Water quality analyses of 105 samples revealed no significant differences among treatments for change in pH ($F_{2,6}$ = 1.93; P = 0.226), specific conductance ($F_{2,6}$ = 0.11; P = 0.902) and acid neutralizing capacity ($F_{2,6}$ = 0.11, P = 0.894; Table 2). Concentrations of ammonia (detection limit: 0.015 mg/liter as N), nitrite and nitrate (detection limit: 0.022 mg/liter as N), and orthophosphate (detection limit: 0.007 mg/liter as P) were at or near the detection limits for many ponds throughout the study; thus, we were unable to analyze these data. Detectable ammonia concentrations (45% of samples) ranged from less than 0.016–0.246 mg/liter and detectable orthophosphate

TABLE 2. Summary of water quality metrics by treatment and time-interval (mean ± SE). Data were collected from 2002–2006 at study ponds in northeastern Oregon.

Treatment	Pretreatment	Posttreatment
pH		
No exclosure (control)	7.67 ± 0.09	7.93 ± 0.13
Partial exclosure	8.19 ± 0.20	7.75 ± 0.09
Full exclosure	7.52 ± 0.11	7.76 ± 0.12
Specific conductance		
No exclosure (control)	311.59 ± 34.86	329.22 ± 21.45
Partial exclosure	368.66 ± 27.09	353.04 ± 24.92
Full exclosure	283.52 ± 26.42	326.05 ± 17.57
Acid neutralizing capacity		
No exclosure (control)	157.89 ± 15.14	168.29 ± 9.65
Partial exclosure	193.40 ± 14.13	177.87 ± 10.92
Full exclosure	153.22 ± 14.30	160.80 ± 7.16

concentrations (67% of samples) ranged from 0.007–0.104 mg/liter. Nitrite plus nitrate levels were below detection limits for all samples.

Discussion

This study represents the first attempt that we are aware of to conduct an experimental manipulation of cattle grazing at whole breeding ponds for an anuran. Despite significant treatment effects on vegetation height, we did not find evidence that full or partial grazing exclosures affected *R. luteiventris* egg mass counts, larval survival, or metamorphic size in the first two or three years after fence installation. The lack of the posttreatment decrease in egg masses in the full exclosure treatment that was seen in the other treatments suggests a possibility that full exclosures change the pond habitat in a way that increases egg mass numbers. This possible effect was not strong enough to reach statistical significance with the number of replicates that we used. We found very low levels of nitrate and orthophosphate, which are compounds associated with livestock use that can have negative effects on other amphibians (Boyer and Grue, 1995; Knutson et al., 2004). Most of our ponds were in riparian areas that have springs and groundwater flow that could potentially dilute nutrients in the ponds. It is also possible that moderate cattle densities, availability of other water sources, and the short grazing season in our study area act to limit the intensity of grazing effects. All of our results should be interpreted cautiously because of low replication and the probability that treatment effects will take longer than two years to fully manifest themselves. In particular, effects of treatment on pond habitats might be cumulative and could only be detected by a change in egg mass numbers which might take several years to emerge. An alternative explanation for the lack of treatment effect on egg mass numbers is that the small riparian buffer provided by the exclosures was inadequate to alter the interaction between cattle and frogs. This might occur if cattle were trampling frogs away from the pond or if terrestrial habitat conditions affect frog populations. Given that *R. luteiventris* is highly aquatic during the season when cattle are present and given the low density of cattle, we view effects away from ponds to be unlikely, but such effects were not addressed by our study.

Other field studies involving *R. luteiventris* have not found a correlation of livestock presence with egg mass numbers in northeast Oregon (Bull and Hayes, 2000) or with the probability of persistence in southeastern Oregon and northern Nevada (Wente et al., 2005). One study (Reaser, 2000) suggested that a truncated age distribution in *R. luteiventris* adults at two Nevada sites could be related to grazing, but the sample size was small, and quantitative data on grazing were not presented. One western Washington study found adult *R. pretiosa* (the closely related Oregon Spotted Frog) used moderately grazed areas more than other portions of a wetland complex (Watson et al., 2003). Parts of that wetland have dense stands of an invasive grass (*Phalaris arundinaceae*); hence, frogs may have responded to reduced stem density (Watson et al., 2003). None of these studies manipulated grazing and, thus, could not necessarily isolate the effects of livestock grazing from other associated effects on *R. luteiventris* populations.

Vegetation cover has been linked with the presence of *R. luteiventris* breeding in Idaho and Oregon ponds (Monello and Wright, 1999; Bull and Marx, 2002), as well as to higher counts of juvenile frogs (Bull and Hayes, 2000). *Rana luteiventris* appear to favor vegetated microhabitats for oviposition (Pearl et al., 2007). These microhabitats may provide basking and feeding sites as well as cover from predators (Bull and Hayes, 2000; Pearl et al., 2005). The treatment effects on vegetation in our study indicate that grazing pressure is strong enough to alter the habitat but did not translate into short-term effects on the frog populations.

Pond construction in agricultural and forested landscapes has added habitat that can be used by *R. luteiventris* (Monello and Wright, 1999; Bull and Hayes, 2000). At present, it is unclear how these ponds compare to habitat types that were historically used by frogs (e.g., oxbows, beaver complexes). Neither our study nor field surveys (Bull and Hayes, 2000; Wente et al., 2005) suggest strong effects of grazing on *R. luteiventris* populations. However, more time is needed to fully assess the effects of the exclosures in our study.

Acknowledgments.—We are grateful to S. Lisle, M. Hall, W. Frye, R. Estes, and the staff at the Whitman District of the Wallowa-Whitman National Forest for logistical support, fence construction, and assistance identifying study sites. We thank J. Alexander, J. Cogswell, L. Inglis, R. McCullough, F. Nolan, A. Sackos, and the Eastern Oregon Miners and Prospectors for their interest and cooperation in the use of ponds. D. Green, C. Rostad, C. Tait, and L. Todd provided helpful discussions in the early phases of the study. This study was funded by the U.S. Fish and Wildlife Service, U.S. Forest Service, and the U.S. Geological Survey's Amphibian Research and Monitoring Initiative. We received state research permits and institutional

animal care approval to conduct this study. Mention of trade names is for informational purposes only and does not constitute endorsement.

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Accepted: 15 July 2008.