Ability of Oregon Spotted Frog (Rana pretiosa) Embryos from Central Oregon to Tolerate Low Temperatures

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More than half of the known remaining populations of the Oregon Spotted Frog (*Rana pretiosa*) are at elevations >1200 m along the Cascade Range and its eastern flank in Oregon (Pearl and Hayes 2005). *Rana pretiosa* in this region typically breed soon after thaw and often lay eggs in water <20 cm deep (Pearl and others 2009). Egg masses in shallow microhabitats experience broad temperature fluctuations, and ice formation on the surface is common (Bull and Shepherd 2003). At >20 oviposition sites around Sunriver in central Oregon (elevation 1270 m), we have observed high survival of *R. pretiosa* embryos after exposure to subfreezing air temperatures and ice cover for up to several days. These observations and the characteristics of oviposition sites across much of the species' range along the Cascade Range suggest that *R. pretiosa* eggs in this region may be more tolerant of low temperatures than previously reported. Information on thermal limits of *R. pretiosa* embryos derives from studies of 1 population in the Fraser River Valley of southwestern British Columbia. The low elevation of this site (approximately 50 m above sea level) and its proximity to the Strait of Georgia and Pacific Ocean make its climate more moderate than much of the extant range of *R. pretiosa*. Licht (1971) reported a lethal minimum, the temperature at which egg survival is <50%, near 6°C for eggs from that site at a range of stable temperature treatments in the laboratory. A subset of embryos survived lower temperatures for up to 8 h, after which trials were terminated (Licht 1971), but data were not presented on presence or timing of developmental abnormalities.

To better understand cold tolerance of *R. pretiosa*, we deployed temperature loggers and quantified embryonic survival at regularly-used oviposition sites around Sunriver and Crosswater, Oregon (UTM: Zone 10T, 624799–624652E, 4855810–4860990N, NAD83). Breeding habitats ranged from a small (0.1 ha) seasonally flooded natural oxbow pond to larger channels and marshes within the Sunriver wetland complex (total surface area about 30 ha in spring). Most sites were anthropogenically created or enlarged and have extensive emergent and submergent vegetation. Our monitoring over the last 10 y in this area has revealed remarkably consistent use of oviposition sites by *R. pretiosa* (contingent on water levels). We monitored 12 oviposition sites in 10 wetlands in 2006, and 8 sites in 6 wetlands in 2007: 2 marshes contained 2 monitored sites each. We used HOBO data loggers (model H08-002) and external probes (TCM6-HA) to record temperature at 1-h intervals. At all study sites, thermistor probes were fastened to horizontal bamboo wands and floated with small Styrofoam blocks to keep probes 3 cm below the surface; these were typically positioned <10 cm from the perimeter of egg mass clusters (range 7 to 30 egg masses/cluster). Comparison of temperatures within and adjacent to egg masses showed negligible temperature differences except during peak daytime temperatures; nighttime lows were nearly identical (Fig. 1). We inspected sites every 1 to 3 d from before oviposition until 1st hatching to track development and detect stranding. We visually estimated embryonic survival at hatching to the nearest 10%. Estimates were conservative and based on our experience monitoring mortality at several hundred egg masses/y at these sites.

To complement field observations, we conducted a simple laboratory trial that exposed embryos to continuous temperatures below those reported as lethal for the lowland population studied by Licht (1971). On 3 April 2008, we collected 2 freshly laid egg masses (stage 2;

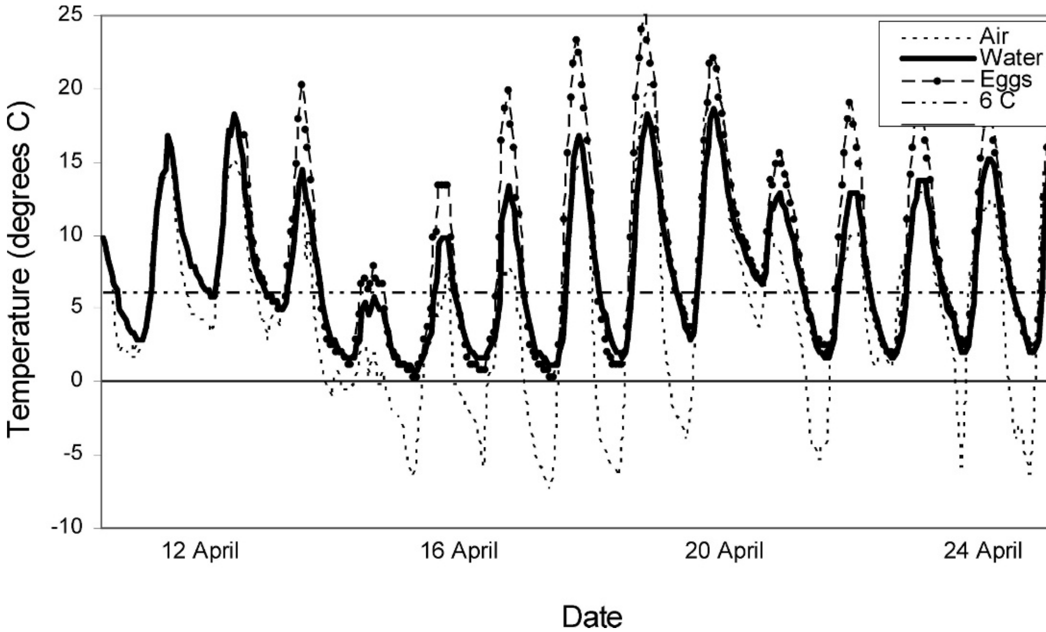

FIGURE 1. Thermograph of a typical *R. pretiosa* oviposition site (South Weir Marsh) in Sunriver, Oregon, from 1st oviposition to 1st hatching (11 April–25 April 2006). Data are from sensors above water surface (air), middle of egg mass cluster (eggs), and water adjacent to egg masses (water). The thermal minimum is 6°C proposed for *R. pretiosa* in lowland British Columbia (Licht 1971).

Gosner 1960) from a site we monitored in 2006 (site FW18; UTM: Zone 10T, 624811E, 4859299N). Each egg mass was placed into a separate 500-ml plastic container with 1 to 2 cm of well water that kept the egg masses hydrated but not submerged. We placed 1 egg mass in a refrigerator that averaged 1.2°C (s = 0.6 °C; range 0.7 to 2.9 °C); the other mass was placed in a 2nd refrigerator that averaged 3.6°C (s = 0.5 °C; range 2.8 to 5.0°C). Egg mass temperatures were measured hourly for the duration of the trial with the same loggers and probes used at field sites. After 1, 2, 3, 4, 10, and 17 d, we removed 10 to 15 embryos (11.9 ± 1.7; $x \pm s$), scored their developmental stage, and inspected them for signs of abnormal development or mortality. We then maintained these embryos in new SOLO™ 2-oz plastic condiment cups with 30 ml of well water at room temperature (17 to 20°C) until larvae reached stage 24 or ceased development.

The thermograph data revealed that water temperatures at oviposition sites dropped below 6°C every night except one night during incubation in both years (Figs. 1 and 2). Moreover, we saw high egg survival to hatching despite appreciable time spent below 6°, 4°, and 2°C (Table 1). In 2007, water temperatures dropped to 2°C or lower at all sites except one, where flowing water moderated temperatures. Air temperatures dropped below freezing most nights and 2 to 5 mm of surface ice was common in the morning. In 2007, egg masses spent approximately half of their incubation periods below 6°C (47 ± 18%; range 43 to 62%). Egg masses consistently warmed above water temperatures during daytime, especially during periods of sunshine, but water and egg mass temperatures were similar at night (Fig. 1). Survival to hatching was >80% at 10 of 12 sites in 2006, and at 6 of 8 sites in 2007; the only observations of lower survival were at sites where receding water level stranded egg masses. Estimates of survival at the 4 oviposition sites that experienced some degree of stranding ranged from 10 to 60%.

In the laboratory, survival to stage 24 was high for embryos maintained at constant temperatures of 1.2°C (92 ± 8%) and 3.5°C (96 ± 5%) through 10 d of cold treatment, and tadpoles that hatched from these eggs appeared normal (Fig. 3). Refrigerated embryos at 1.2°C

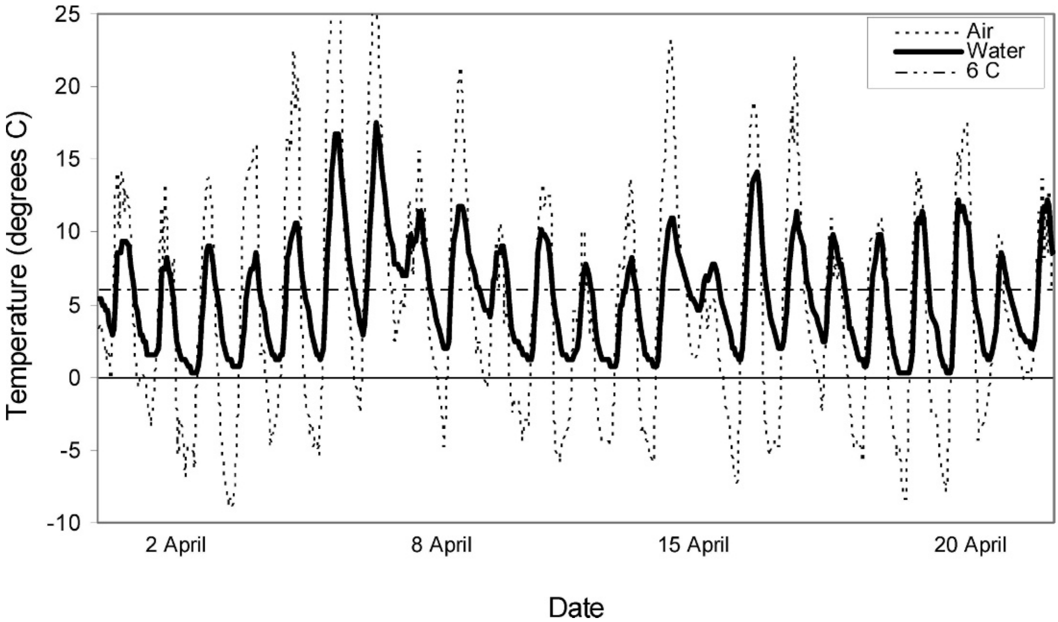

FIGURE 2. Thermograph for ''cold'' *R. pretiosa* oviposition site (Duck Pond) in Sunriver, Oregon, from 1st oviposition to 1st hatching (31 March–22 April 2007). Data are from sensors above water surface (air) and water adjacent to egg masses (water). The thermal minimum is 6°C proposed for *R. pretiosa* in lowland British Columbia (Licht 1971).

developed to stage 7 by day 17 (approximately 400 h). Embryos at 3.5°C developed to stage 8–9 by day 17. When the embryos were moved to room temperature after 17 d, 2 of 14 embryos from the 1.2°C treatment and 4 of 15 embryos from the 3.8°C treatment survived to stage 24 and appeared normal. None of the remaining embryos in these treatments developed past stage 18; most exhibited developmental abnormalities such as failure of the blastopore to close, stubby and lumpy bodies, or gross body asymmetries.

The laboratory trial showed that *R. pretiosa* embryos in our study area can tolerate at least 10 d of continuous water temperatures below the 'lethal minimum' reported for the lowland population studied by Licht (1971). We found no field evidence of temperature-related mortality or abnormal development despite daily exposure to temperatures below 6°C that totaled >200 h. Indeed, one group of 7 egg masses from a shady site showed normal development and high survival (>80%) over a protracted incubation period (about 30 d) and cumulative exposure of >400 h to water temperatures <6°C (Fig. 2). Our field observations and refrigeration trial included early embryonic stages that are the most sensitive to thermal extremes in other anurans (Brown 1967; Herreid and Kinney 1967).

TABLE 1. Hatching rates, incubation periods, and cumulative hours below 6°, 4°, and 2°C at *R. pretiosa* oviposition sites around Sunriver, Oregon.

		Incubation period (days)			Hours <6°C			Hours <4°C			Hours <2°C		
Year	HR >80%[a]	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
2006	10 of 12 [b]	14.8	15.0	10–17	118.1	132	13–178	73.7	78.5	0–123	24.9	22.5	0–72
2007	6 of 8 [b]	20.5	19.0	16–30	237.5	278.5	29–404	165.9	206.5	0–275	92.0	102.0	0–179

[a] Numbers equal number of sites.
[b] Hatching rates <80% were observed only when egg masses were stranded by receding water levels. Mean estimated hatching rate across all sites and years was >73% (range 10–90%).

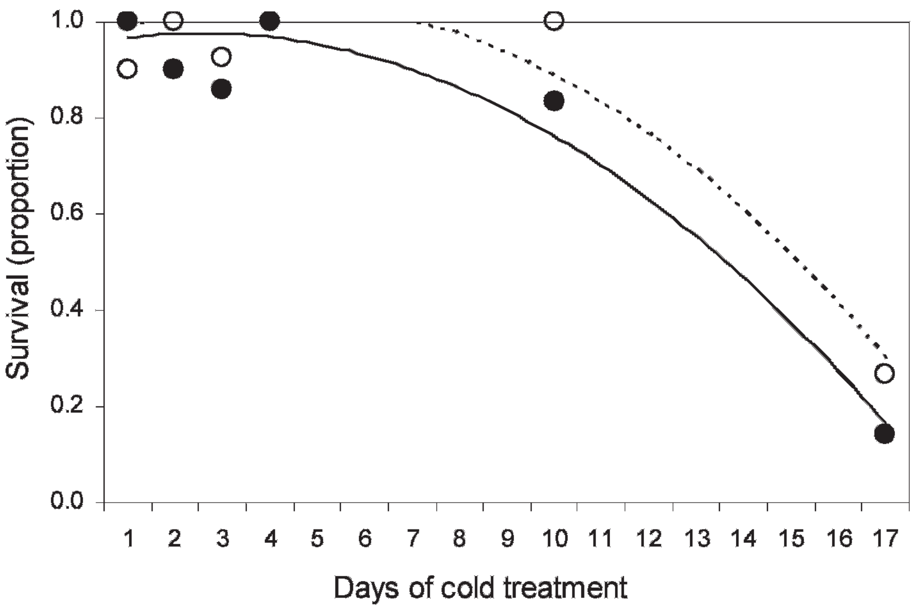

FIGURE 3. Survival of *R. pretiosa* embryos to stage 24 after refrigeration at 1.2°C (solid circles, heavy line) or at approximately 3.5°C (open circles, dashed line). Embryos in each temperature treatment were from 1 egg mass. Survival was based on 10–15 embryos removed from respective refrigerators on each of the indicated days (1, 2, 3, 4, 10, and 17) after trial initiation.

Early studies investigated temperature tolerances of anurans by exposing eggs to a range of constant temperatures in controlled laboratory settings (Moore 1939; Johnson 1965; Herreid and Kinney 1967; Licht 1971). Johnson (1965) and Licht (1971) used nearly identical laboratory methods and concluded that embryos of *R. luteiventris* (formerly *R. pretiosa luteiventris*; Green and others 1996) and *R. pretiosa*, respectively, would not survive and develop at constant temperatures below 6°C. Data from our study of *R. pretiosa*, and from Bull and Shepherd (2003) for *R. luteiventris*, however, show that embryos of both species survive regular exposure to temperatures <6°C in central and eastern Oregon.

Among ranid frogs, many traits, such as growth and longevity (Berven 1982; Miaud and others 1999), UV sensitivity (Marquis and Miaud 2008), and acid tolerance (Räsänen and others 2003), are known to vary geographically and with elevation. Substantial population variation in embryonic cold tolerance can be expected for spotted frogs, which occur across elevation spans of >1500 m. Because egg mass temperatures vary daily across a considerable range and inter-population variation of cold tolerance has not been tested, we advise caution in extrapolating from laboratory results based on constant temperature treatments to field survival in early-breeding frogs such as *R. pretiosa*.

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