Timing of Trematode-Related Malformations in Oregon Spotted Frogs and Pacific Treefrogs

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TIMING OF TREMATODE-RELATED MALFORMATIONS IN OREGON SPOTTED FROGS AND PACIFIC TREEFROGS

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Large numbers of malformed amphibians have been reported from across North America over the past decade (Helgen and others 1998; Sessions and others 1999; Meteyer and others 2000; Johnson and others 2002). Although sporadic reports of gross malformations, including extra limbs, date back to at least the 18th century (Bland-Sutton 1890; Van Valen 1974), there is growing evidence that recent occurrences are more widespread, more severe, and affect a larger percentage of some populations than documented historically (Hoppe 2003). A number of hypotheses have been proposed as possible causes of deformed amphibians, including chemical pollution (Ouellet and others 1997; Gardiner and Hoppe 1999), ultraviolet-B radiation (Blaustein and others 1997; Ankley and others 2000), predation (Bohl 1997; Johnson, Lunde, Haight, and others 2001), and trematode parasites (Sessions and Ruth 1990; Johnson and others 1999).

In the western United States, trematode infection has been identified as a widespread cause of amphibian deformities (Sessions and Ruth 1990; Johnson and others 1999; Johnson, Lunde, Ritchie, and others 2001). Laboratory infections of larval Pacific treefrogs (*Pseudacris regilla*) and western toads (*Bufo boreas*) using the digenetic trematode *Ribeiroia ondatrae* caused high levels of malformation and mortality (Johnson and others 1999; Johnson, Lunde, Haight, and others 2001). These malformations were similar to those observed at field sites, including extra, missing, and malformed limbs, and resulted from disruption of the positional relationships of cells in the developing limb buds (Stopper and others 2002). Field investigations in 5 western states indicated that *Ribeiroia* sp. infection was the strongest predictor of high levels of amphibian limb malformations, and trematode-associated malformations were found in 11 of 12 amphibian species examined (Johnson and others 2002). No malformations were recorded in the Oregon spotted frog (*Rana pretiosa*), despite the co-occurrence of *Ribeiroia* sp. and *R. pretiosa* at 5 sites. Moreover, few malformations (<0.5%) have been observed among more than 1300 juvenile and 4000 adult *R. pretiosa* from 6 sites in Central Oregon between 1999 and 2003 (JB, unpubl. data).

In the summer and fall of 2002, we found a high frequency (22%, $n = 59$) of malformed *R. pretiosa* metamorphs in the Nature Center Pond, a shallow, artificial pond in Sunriver, Oregon (elevation 1384 m). Several *P. regilla* with supernumerary limbs and other malformations typical of *Ribeiroia* sp. infection (for example, Johnson and others 2002) were also captured during pond surveys.

We noted early in the season that the Nature Center Pond had an unusually high population of large planorbid snails (*Planorbella* sp.), the required intermediate host of *Ribeiroia* sp. We estimated the prevalence of patently infected snails on 4 dates during the summer by isolating snails overnight in transparent plastic cups containing a small amount of water. Infected snails released between about 20 and 1200 *Ribeiroia* sp. cercariae per night. When these cercariae were placed with a tadpole, they adhered quickly to the tadpole and crept systematically across the skin to the inguinal region (area around the base of the hind limbs) where they encysted as metacercariae.

We obtained *Ribeiroia* sp. cercariae from 37 of 281 snails (13.2%) collected between 6 June and 24 September 2002 (Fig. 1). The proportion of snails actively releasing *Ribeiroia* sp. apparently peaked in August with a subsequent decline in September and October.

Five of 123 *P. regilla* larvae and metamorphs collected from the Nature Center Pond between

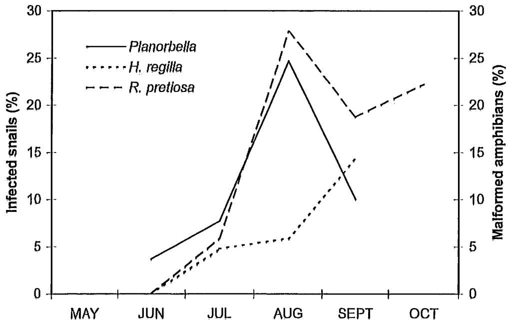

FIGURE 1. Prevalence of snail (*Planorbella* sp.) infection and amphibian (*Pseudoacris regilla* and *Rana pretiosa*) malformations in Nature Center Pond, Oregon, in 2002.

29 June and 28 September exhibited malformations: 2 with 1 or more extra hind limbs (polymelia), 1 with polymelia and hind limb skin webbing (cutaneous fusion), 1 with cutaneous fusion, and 1 with a bony triangle in a hind limb (taumelia). We found 13 malformed *R. pretiosa* among 59 metamorphs collected during August, September, and October. Malformations included extra hind foot digits (polydactyly), cutaneous fusion across a hind limb joint, and extra hind limbs. The most severely malformed metamorph exhibited a bony triangle in the tibiofibula of the left hind limb, 2 missing digits on the left hind foot, 2 incompletely developed extra limbs on the dorsal margin of the pelvis, and an extra digit on the right hind foot (Fig. 2). Dissection of this individual yielded 69 *Ribeiroia* sp. metacercariae, which were concentrated around the inguinal region and tail resorption site. Some metacercariae were in close association with malformed tissue (Fig. 3).

We also investigated 3 additional artificial ponds within 10 km of Sunriver: Bullfrog Pond (BFP) and North Driving Range Pond (NDR) in the community of Crosswater, and Borrow Pit Pond 4 (BPP4) on Bureau of Land Management land south of Sunriver. All ponds supported planorbid snails but *Ribeiroia* sp. prevalence was relatively low, and none of these ponds yielded malformed frogs (Table 1).

This represents the 1st report of trematode-associated malformations in *R. pretiosa*. We suggest that the timing of exposure to parasites explains why some species, such as *R. pretiosa*, rarely exhibit trematode-induced malformations, whereas others, such as *P. regilla*, seem to be particularly sensitive.

FIGURE 2. Malformed *Rana pretiosa* showing supernumerary hind limbs and skin webbing.

The probability that *Ribeiroia* sp. infection in amphibian larvae leads to abnormal development is related to both dosage and timing of parasite exposure (Johnson and others 1999;

FIGURE 3. Detail of malformed *Rana pretiosa* limb showing trematode cycts in inguinal area.

TABLE 1. Parasite infection of snails and amphibian malformations at 4 ponds in central Oregon in 2002.

				Rana pretiosa			*Pseudacris regilla*		
Pond[1]	Snails collected	Snails infected	% infected	*n*	Number malformed	% malformed	*n*	Number malformed	% malformed
NCP	281	37	13.2	59	13	22.0	127	4	3.1
NDR	50	1	2.0	1	0	0.0	83	0	0.0
BFP	83	2	2.4	42	0	0.0	10	0	0.0
BPP4	241	2	0.8	0	0		0	0	

[1] NCP = Nature Center Pond, NDR = North Driving Range Pond, BFP = Bullfrog Pond, and BPP4 = Borrow Pit Pond 4.

Stopper and others 2002). Amphibians exposed late in development, beyond the window of limb growth and differentiation, are unlikely to develop malformations. This helps explain why, at ponds that support *Ribeiroia* sp., metacercariae can generally be recovered from all amphibians, regardless of whether they are deformed. The types of malformations induced are also influenced by the timing of parasite exposure. Early infections lead to severe, proximal abnormalities (for example, extra limbs), whereas infections occurring later in development are associated with distal, less severe malformations (for example, extra digits).

Pseudacris regilla, the anuran species most commonly reported with malformations in the western United States, breeds continuously with a breeding season of ≥2 months. Consequently, a broad range of developmental stages is present over an extended time period, dramatically increasing the likelihood of phenological overlap with cercarial shedding by *Ribeiroia*-infected snails and subsequent induction of limb malformations among emerging frogs. Johnson, Lunde, Ritchie, and others (2001) reported that the incidence of trematode-associated malformations in larval *P. regilla* increased throughout the season, often reaching malformation frequencies of ≥50%.

Unlike *P. regilla, R. pretiosa* breeds explosively soon after ponds become ice-free in the spring (Licht 1971). Breeding typically lasts a few days, with eggs generally laid in shallow, weedy sites that are warmed rapidly by the sun (Licht 1971; Hayes and Engler 1999). Early development tends to be rapid and nearly synchronous. In both the current study and unpublished data from California (Johnson and Lunde 2003), the prevalence of *Ribeiroia* sp. infection in snails peaked in late August, after the normal period of limb development in *R. pretiosa.* Thus, at most sites and in most years, *R. pretiosa* probably escapes heavy levels of *Ribeiroia* sp. infection during the critical stages of limb development, thereby explaining the typical rarity of malformations in this species. However, local or regional factors that delay metamorphosis in *R. pretiosa* such as delayed oviposition and/or slow development resulting from unusually cold temperatures, could increase the potential for phenological overlap with infected snails and thus promote exposure to *Ribeiroia* sp. and the production of limb malformations.

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