TWENTY

Parasite Infection and Limb Malformations: A Growing Problem in Amphibian Conservation

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Over the last two decades, scientists have become increasingly concerned about ongoing trends of amphibian population decline and extinction (Blaustein and Wake, 1990, 1995; Phillips, 1990; Pechmann et al., 1991; Wake, 1998). Parasitic pathogens, including certain bacteria, fungi, viruses, and helminths (see also Sutherland, this volume), have frequently been implicated as causes of gross pathology and mass die-offs, often in synergism with environmental stressors (Dusi, 1949; Elkan, 1960; Elkan and Reichenbach-Klinke, 1974; Nyman, 1986; Worthylake and Hovingh, 1989; Carey, 1993; Blaustein et al., 1994b; Faeh et al., 1998; Berger et al., 1998; Morell, 1999; Kiesecker, 2002; Lannoo et al., 2003). The effects of human activity on the epidemiology of endemic and emerging diseases remain largely unknown (Kiesecker and Blaustein, 1995; Laurance et al., 1996).

More recently, malformed amphibians have been reported with increasing frequency in several parts of North America. Since 1992, severe limb abnormalities, including extra, missing, and malformed limbs, have appeared in dozens of species from diverse habitats, including several species in marked decline (Ouellet et al., 1997a; Helgen et al., 1998; Burkhart et al., 1998; NARCAM, 1999). While it is unlikely that these abnormalities have been a major source of historical amphibian population declines, they may represent an emerging threat, particularly if they are increasing in frequency (Wake, 1998; Hoppe, 2000). A generally low (0–5%) baseline rate of abnormalities, due to mutation, trauma, and developmental errors, may be expected in most amphibian populations (Martof, 1956b; Meyer-Rochow and Asashima, 1988; Zaffaroni et al., 1992; Tyler, 1998), but many recent accounts consistently document frequencies of 15% or greater (e.g., Hoppe and Mottl, 1997; Johnson et al., 2001b). Limb malformations are widely suspected to impair the survival of affected individuals, and some researchers have recorded corresponding mass die-offs (Gardiner and Hoppe, 1999).

The potential danger of these malformations to amphibians and possibly other vertebrate species depends, in part, on the agent(s) responsible. Among those currently under investigation are ultraviolet radiation (Blaustein et al., 1997; Ankley et al., 1998), pesticides (Ouellet et al., 1997a; Burkhart et al., 1998; Fort et al., 1999a; Kiesecker, 2002), retinoids (Gardiner and Hoppe, 1999), and parasites (Sessions and Ruth, 1990; Johnson et al., 1999; Sessions et al., 1999). The recent and widespread nature of the malformations has focused the investigation on the direct and indirect impacts of human activity. One of the prime suspects in the East and Midwest has been water contaminants. Ouellet et al. (1997a) documented high rates of limb malformations in amphibians from agricultural ponds in southeastern Canada; laboratory researchers in Minnesota have found that water from affected ponds causes some malformations in African-clawed frog *(Xenopus laevis)* embryos and larvae. Identification of the active compounds and their role in amphibian limb malformations in the field remains under investigation (Fort et al., 1999b). The wide geographic range in North America with reports of abnormal amphibians suggests multiple causes are responsible, either interactively within ponds, independently among ponds/regions, or both.

In the western United States many of the reported sites have been linked directly to intense infections of a little-studied trematode (*Ribeiroia ondatrae*; Johnson et al., 1999, 2001b, 2002; Figs. 19-3, 19-4, and 19-7 in Sutherland, this volume). In laboratory infections, *Ribeiroia* parasites target the developing limb tissue of anuran larvae, inducing high rates of limb malformations strikingly similar to those recorded in field reports (Johnson et al., 1999; 2001a). Laboratory-infected larvae also suffer greater mortality than uninfected conspecifics. At this point, the impacts of *Ribeiroia* infection and the resulting limb abnormalities on natural amphibian populations are still poorly understood. Concerns about the potential role of human activity in increasing the range or abundance of *Ribeiroia* have yet to be addressed. In other parts of the world, however, human impacts have dramatically influenced the prevalence and infection intensity of a variety of trematodes, often with devastating consequences on related host species.

Here we evaluate two issues concerning *Ribeiroia* infection and amphibian limb malformations. First we ask if parasite-induced malformations in amphibians have increased in frequency, severity, or geographic prevalence. To this end, we examine (1) published accounts of malformed amphibians in the western United States; (2) the frequency of abnormalities

in vouchered museum specimens; and (3) the historic range and host records of *Ribeiroia*, as recorded in the parasitology literature. In the second section, we assume that an increase in the above-stated attributes has occurred, and we investigate the most likely causal factors. Of particular concern are human impacts on trematodes, and we review the various mechanisms of influence and evaluate their relevance to *Ribeiroia* epidemiology.

Trematode Infections and Amphibian Malformations

Digenetic trematodes are a diverse group of exclusively parasitic flatworms common within most vertebrate groups. Of great interest to many parasitologists are trematodes' complex life cycles, which typically involve two or more hosts (see Sutherland, this volume). Sessions and Ruth (1990) suggested a connection between trematode infection and abnormal amphibians after observing a close association between metacercariae *(Manodistomum syntomentera)* and limb malformations in a California pond. The abnormalities included extra and deformed hindlimbs and affected two amphibian species: Pacific treefrogs *(Pseudacris regilla)* and Santa Cruz long-toed salamanders *(Ambystoma macrodactylum croceum)*. As an indirect test of their hypothesis, Sessions and Ruth (1990) implanted inert resin beads of a similar size to metacercariae in the developing limbs of African-clawed frog and spotted salamander *(Ambystoma maculatum)* larvae. Twenty percent of the treated animals six percent of the controls exhibited minor limb anomalies. This result suggests that mechanical disruption of limb development caused by the parasitic cysts might be contributing to the limb abnormalities observed in the field (Sessions and Ruth, 1990).

In an independent study, we reported a field correlation between infection with a different trematode species and sites with high rates (10–45%) of amphibian limb malformations (Johnson et al., 1999; Fig. 20-1). Over a two-year period, we inspected 15,000 amphibians from 35 ponds in northern California. Malformations were recorded only from sites that supported the trematode *Ribeiroia* and its snail host, *Planorbella tenuis*. To test the hypothesis that *Ribeiroia* infection caused the observed malformations, we exposed laboratory-raised Pacific treefrog larvae to specific numbers of *Ribeiroia* cercariae isolated from infected snails. The infections had dramatic effects, and severe hindlimb deformities similar to those observed in the field were recorded in all *Ribeiroia* treatments. The frequency of abnormalities ranged from 70% (light infection) to 100% (heavy infection), whereas the number of hindlimbs ranged from zero to as many as eight. Larvae either exposed to a second species of trematode (*Alaria mustelae*; Fig. 19-8A in Sutherland, this volume) or not exposed to parasites did not exhibit abnormalities and had high survivorship. Subsequent experiments with western toads *(Bufo boreas)* have replicated and expanded these results. Using the same protocol, we again induced high rates of limb deformities, which in toads included extra, missing, and malformed forelimbs as well as hindlimbs (Johnson et al., 2001a).

Among trematodes with complex life cycles, species that reduce intermediate host fitness are reported with increasing frequency (reviewed by Moore and Gotelli, 1990; Kuris, 1997; Poulin, 1998). As the completion of these worms' life cycles depends on predation of the intermediate host by the definitive host (trophic transmission), selection favors traits impairing the intermediate host's ability to evade predation. In a classic example, Bethel and Holmes (1977) documented an amphipod that, when infected with a species of acanthocephalan, swims erratically near the water's surface and is more frequently consumed by foraging ducks, which are the parasite's definitive host. In a similar fashion, we suspect malformed limbs increase the likelihood that an infected frog will be eaten by a definitive host of *Ribeiroia*, which include muskrats, waterbirds, and raptors (Fig. 20-2). As with many other impairing parasites, *Ribeiroia* exhibits intensity-dependent pathogenicity—greater infections cause higher rates of limb malformations (Kuris, 1997; Johnson et al., 1999). Unlike most other parasites, *Ribeiroia* induces profound structural changes in its host's body plan. Changes in a host's morphological development (such as the number of limbs) are unusual among the many other accounts of parasite manipulation, which often report changes in the intermediate host's behavior (Moore, 1983, 1984a,b; Lafferty and Morris, 1996) or physical conspicuousness (Camp and Huizinga, 1979; Bakker et al., 1997). The mechanism through which *Ribeiroia* affects limb development is not known, but probably involves mechanical disturbance of the developing limb field (Sessions et al., 1999), secretion by the parasite of a limb growth factor (e.g., retinoic acid, sonic hedgehog, FGF-8), or a combination of the two (Johnson et al., 1999).

FIGURE 20-1 Pacific treefrog *(Pseudacris regilla)* with extra hind limbs.

Recently, limb deformities associated with *Ribeiroia* infection were recorded in frogs, toads, and salamanders from across the western United States (Johnson et al., 2002; Fig. 20-3; Table 20-1). In a survey of more than a hundred ponds throughout California, Oregon, Washington, Idaho, and Montana, Johnson et al. (2002) reported a clear association between *Ribeiroia* infection and amphibian deformities; approximately 90% of the sites where limb malformations were observed supported *Ribeiroia*. No association between pesticide presence and amphibian malformations was detected. The abnormalities, which ranged in frequency from 1–90%, included missing limbs, extra limbs, skin webbings, and bony triangles. Affected sites were diverse, encompassing eutrophic stock ponds, mitigation ponds, agricultural run-off

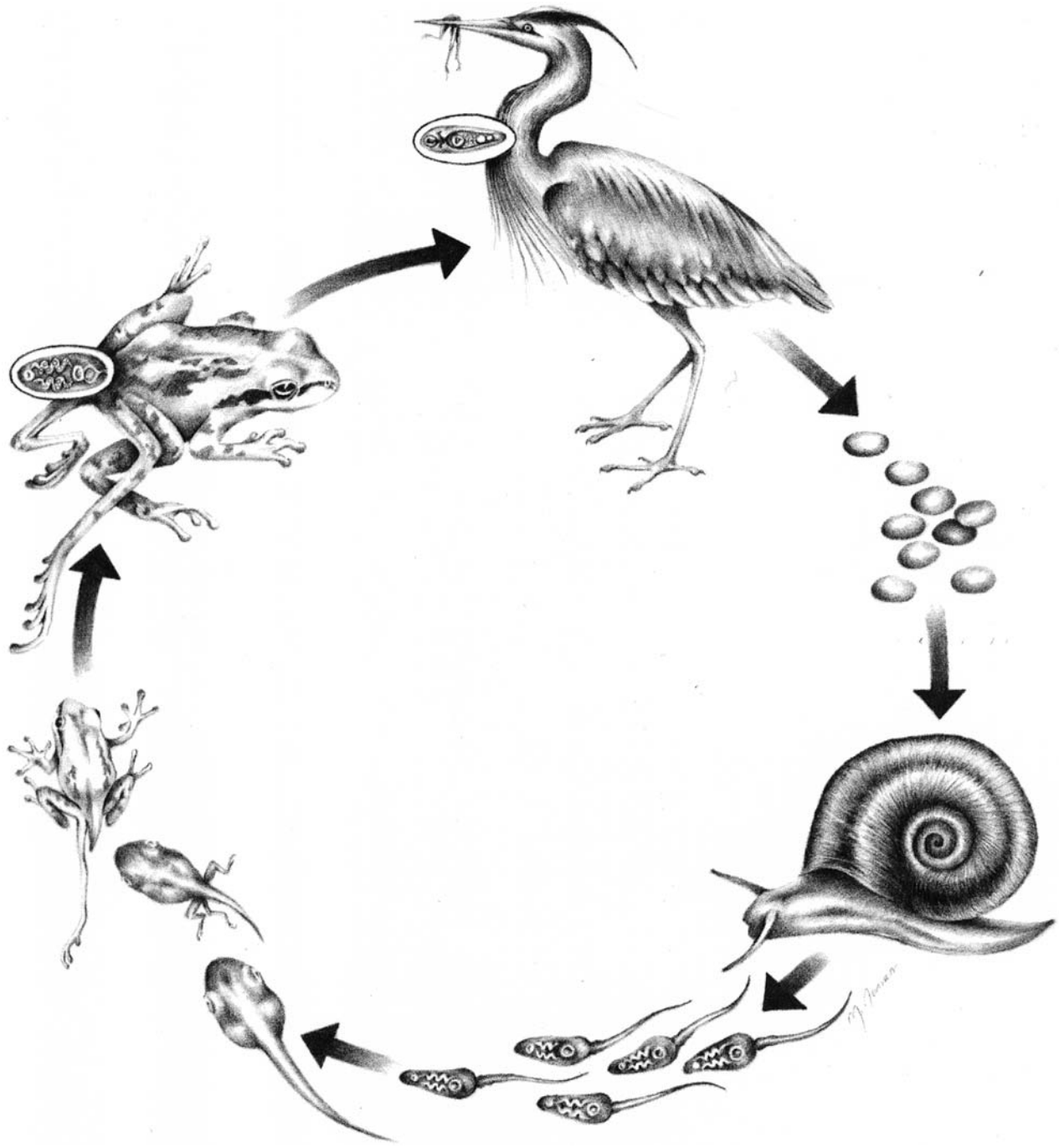

FIGURE 20-2 The life cycle of *Ribeiroia ondatrae*. Depicted hosts are as follows: first intermediate host, planorbid snail (*Planorbella* sp.); second intermediate host, Pacific treefrog *(Pseudacris regilla)*; final host, great blue heron *(Ardea herodias)*.

canals, and montane lakes, but almost all shared a common feature: the presence of *Ribeiroia* and one of its requisite snail hosts.

Are Parasite-Induced Deformities on the Rise?

Since 1995, it has been suggested frequently that amphibian malformations are more severe, more widespread, and affecting a greater percentage of a given population than in the historical record. Hoppe (2000; this volume), after examining museum vouchers and resurveying historic field sites, effectively concluded that malformations in Minnesota northern leopard frogs *(Rana pipiens)* have become more common and more severe since 1993. Drawing upon recent studies of *Ribeiroia* infection and amphibian deformities, we examine the uniqueness of the current malformation phenomenon in the western United States, narrowing our focus to a single cause (parasite

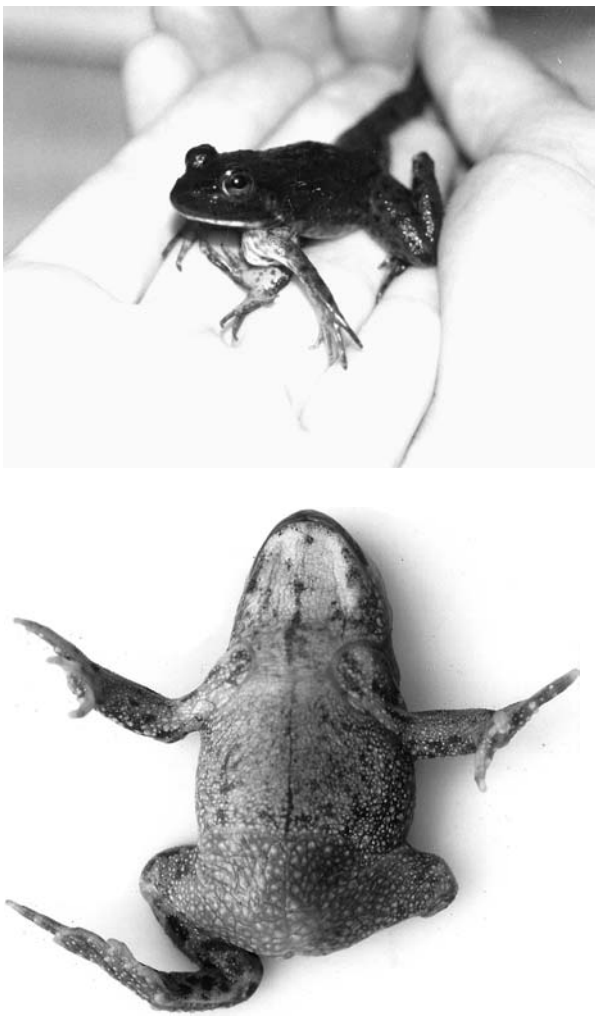

FIGURE 20-3 A bullfrog *(Rana catesbeiana)* from California with two extra forelimbs (above), and a western toad *(Bufo boreas)* from Oregon with a partially missing hindlimb (below).

TABLE 20-1

Conservation Status of Amphibian Species with Limb Abnormalities in the Western United States

Species	*Malformation Type*	*Reported States*	*Species of Concern Listing*[a]
Ambystoma macrodactylum	EX	MT	
A. macrodactylum croceum	EX	CA	CA[b]
Bufo boreas	MIS, MAL, EX	CA, OR, WA	OR, WA
Pseudacris regilla	EX, MAL, MIS	CA, OR, WA, MT	
Rana aurora aurora	MAL	OR	CA, OR, WA
R. cascadae	MAL, EX	OR	CA, OR, WA
R. catesbeiana	MIS, EX, MAL	CA	
R. luteiventris	MAL	WA, MT	WA
R. pipiens	EX	AZ	CA, AZ, OR, WA, MT

[a] Federal or state listing of Species of Concern as offered by the California Department of Fish and Game, the Oregon Department of Fish and Wildlife, the Washington Department of Fish and Wildlife, the Idaho Department of Fish and Game, Arizona Game and Fish, and Montana Fish, Wildlife, and Parks.

[b] Federally listed as Endangered.

NOTE: The types of observed limb abnormalities are abbreviated in the second column and are presented in the approximate order of observed occurrence for each species: EX = extra limb(s); MAL = malformed limb(s); MIS = missing limb(s). The western states in which these species have been recorded with limb abnormalities are also presented (California, Oregon, Washington, Montana, and Arizona). Data adapted from Johnson et al. (1999, 2001b, 2002), Sessions et al. (1999), and Sessions and Ruth (1990).

infection) and to a confined array of abnormalities (limb malformations). This approach offers several advantages over attempts to evaluate the national issue at large. First, it limits the geographic area to a particular region, and the western United States has been an active area of amphibian research for nearly a century. Second, *Ribeiroia* infection is correlated strongly with the presence of amphibian abnormalities in the West, potentially reducing the number of causes under consideration. Finally, one of the most common malformations associated with *Ribeiroia* infection—supernumerary limbs—is highly noticeable and has a rich historical literature. Operating with the null hypothesis that limb deformities in western amphibians (and the presence of *Ribeiroia* metacercariae) have not changed in frequency or prevalence, we use three lines of historical information to examine this notion: (1) historical reports of amphibian limb deformities, (2) abnormality rates in museum voucher specimens, and (3) previous host records and documented range information for *Ribeiroia*. While we recognize the substantial limitations of each data set and their inability to provide a rigorous test of the hypothesis, they may be suggestive of general trends.

Historical Reports

Setting 1990 as our dividing line, we classify reports published prior to 1990 as "historical" and all subsequent publications as "recent." Among the recent reports are five studies offering information on malformed amphibians in the West. Taken together, these reports document severe limb abnormalities in eight species from 47 sites across California, Arizona, Washington, Montana, and especially Oregon (Table 20-1). The majority of the limb abnormalities involve supernumerary limbs (1–12 extra), but also include large numbers of missing limbs, skin webbings, and malformed limbs. The frequency of affected amphibians at a given site varied dramatically, ranging from 1–90%.

Although not new in the western United States, amphibian limb malformations are uncommonly reported. Reports date back as far as 1899 and have steadily increased through the current decade. This increase may be due to increases in parasite abundance, the amount of amphibian research, or the number of available scientific journals. In total, our survey yielded ten independent publications on amphibian limb abnormalities. The reports offer accounts from four western states, encompassing only ten sites and five species (Table 20-2). With two exceptions, all describe extra-legged frogs (1–3 extra). Cunningham (1955)

TABLE 20-2
Historic Reports of Amphibian Limb Abnormalities in the Western United States

Species	*Year*	*State*	*Abnormality*[a]	*Frequency*[b]	*Citation*
Bufo boreas	1896	OR	Extra fore	1	Washburn (1899)
B. boreas	1920	CA	Extra hind	1	Crosswhite and Wyman (1920)
B. boreas	1923	CA	Missing digits, feet, limbs	50%	Storer (1925)
Rana aurora draytonii	1935	CA	Extra hind; missing fore	2	Cunningham (1955)
R. catesbeiana	1948	CA	3 extra fore	1	Pelgen (1951)
R. catesbeiana	1960	CA	3 extra hind	1	Ruth (1961)
Pseudacris regilla	1958–1961	MT	1–2 extra hind	21% (86)	Hebard and Brunson (1963)
R. boylii	1962	CA	2 extra hind	1	Banta (1966)
P. regilla	1967	WA	1–2 extra hind	some	Miller (1968)
P. regilla	1981	ID	1–2 extra hind	22% (54)	Reynolds and Stephens (1984)

[a] All abnormalities involve the limbs, either forelimbs or hindlimbs.
[b] The number of described individuals or the frequency and (sample size).

notes a California red-legged frog *(R. draytonii)* with a complete missing limb, and Storer (1925) describes a series of adult western toads *(B. boreas)* with partially missing limbs and feet. Storer (1925) estimated that approximately 50% of the toads were affected and, based on the recent nature of their abnormalities, suspected injury from a lawn mower was responsible.

Storer's report excluded, six of the nine remaining historic accounts document only one or two abnormal specimens and provide no mention of frequency. Three sites however, offer more interesting and reliable comparisons to recent material. All discuss Pacific treefrogs with supernumerary hindlimbs, which also accounts for most of the recent abnormality reports linked to *Ribeiroia* infection in the West (Johnson et al., 1999, 2002). Miller (1968), unfortunately, provided no quantitative frequency data for the "several" five-legged treefrogs and the "some" that had six limbs. Reynolds and Stephens (1984) reported an abnormality rate of 22% in metamorphic treefrogs from an Idaho pond; all frogs examined had one or more extra limbs (n = 54). The most interesting data, however, come from Jette Pond in western Montana (Fig. 20-4A). Between 1958–61, Hebard and Brunson (1963) consistently found extra-legged treefrogs at a rate of 20–25%. In a recent re-survey of Jette Pond, Johnson et al. (2002) found that approximately 50% of the treefrog larvae and metamorphic frogs exhibited severe malformations (n = 215). Correspondingly, high intensities of *Ribeiroia* infection were universal within affected frogs. Although the frequency of abnormalites is known to vary across a season, recent collections were made within one week of historic sampling dates. Undoubtedly, more data are needed from the Jette site, but these preliminary results suggest a local increase in the frequency of limb malformations. Additionally, two of the four species breeding at this site in the 1950s, Columbia spotted frogs *(R. luteiventris)* and northern leopard frogs are no longer present (Hebard and Brunson, 1963; Anderson, 1977). The fourth species, long-toed salamanders *(A. macrodactylum)*, which Hebard and Brunson (1963) reported as unaffected by limb anomalies, recently has been recorded at Jette Pond with supernumerary limbs at frequencies of 10% (J. Werner, personal communications).

Museum Specimens

Within the collections of the San Diego Natural History Museum (SDNHM) and the Los Angeles County Museum of Natural History (LACMNH), limb abnormalities in Pacific treefrogs are minor and of low frequency. These collections include specimens from 36 counties in California, Oregon, and Washington, as well as vouchers from Canada and Mexico. Of the 1,328 frogs examined at LACMNH and the 658 at SDNHM, 1.74% and 0.91%, respectively, suffered limb abnormalities. Digit amputations, which are confounded by the common marking practice of toe-clipping, were not included in this calculation. Sixty percent of the abnormalities involved missing or partially missing limbs. Three frogs (10% of the abnormalities) with a split digit (minor polydactyly) were also noted. We found no supernumerary limbs, skin webbings, or other serious limb malformations. While the inclusion of collections from other museums would greatly supplement this line of study, we noticed collection biases with respect to life history stage (adult over larval) and habitat (lotic over lentic waters), which questions the utility of this type of evidence. In both historic and recent literature, abnormalities are most commonly recorded in metamorphosing frogs from pond habitats.

Parasitology Literature

Although Johnson et al. (2002) documented *Ribeiroia* in nearly a dozen species of frogs, toads, and salamanders, we found no previous amphibian host records for the western United States (e.g., Ingles, 1933a,b, 1936; Turner, 1958b; Frandsen and Grundmann, 1960; Lehmann, 1960; Pratt and McCauley, 1961; Waitz, 1961; Koller and Gaudin, 1977; Moravec, 1984; Goodman, 1989; Goldberg et al., 1996c, 1998b). In fact, as far as we can determine, *Ribeiroia* has never previously been recorded in a wild amphibian, despite extensive volumes dedicated to amphibian helminths (Brandt, 1936b; Walton, 1947; Smyth and Smyth, 1980; Prudhoe and Bray, 1982; Brooks, 1984; Aho, 1990; Andrews et al., 1992).

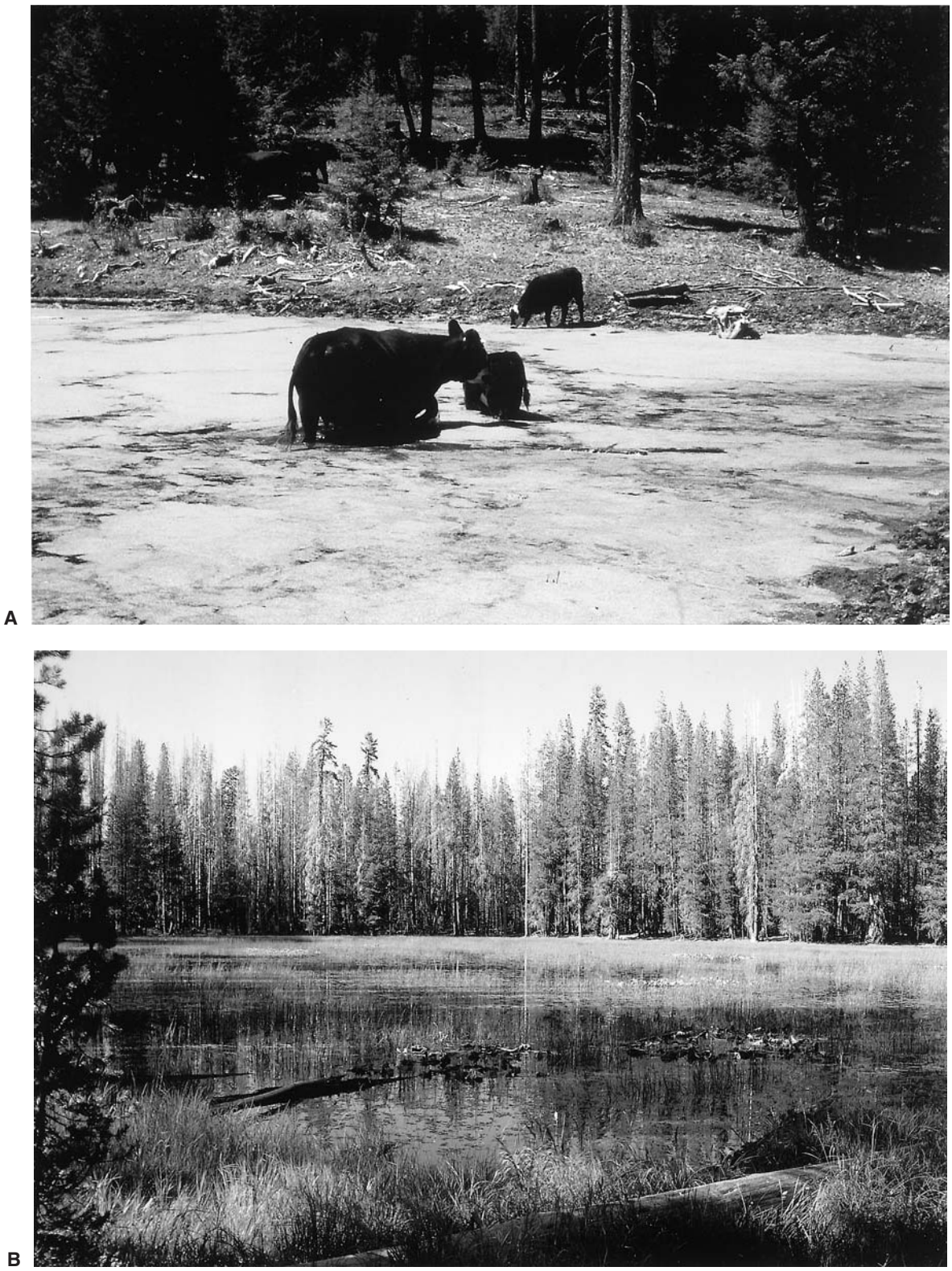

FIGURE 20-4 **(A)** Jette Pond in western Montana exemplifies a culturally eutrophic stock pond in which dense populations of helisome snails are found. **(B)** In a naturally eutrophic pond in Lassen National Park, California, helisome snail numbers are low.

However, Beaver (1939) and Riggin (1956) did conduct experimental infections with several anurans and salamanders in the Midwest and Puerto Rico, respectively, suggesting amphibians historically have served as suitable hosts for *Ribeiroia*. Unfortunately, most helminth surveys focus on the adult parasites of frogs, decreasing the likelihood *Ribeiroia* metacercariae would be noticed. Additionally, trematode taxonomy depends primarily on adult morphology, and larval stages (meta- and mesocercariae), when recorded, are rarely identified.

Caveats and Conclusions

Based on our review of the historic literature on amphibian abnormalities, limb malformations are not new to western amphibians. They are, however, uncommonly reported, especially in light of the amount of amphibian research in the West and on Pacific treefrogs—the most commonly affected species (e.g., Brattstrom and Warren, 1955; Jameson 1956b, 1957; Johnson and Bury, 1965; Altig and Brodie, 1968; White and Kolb, 1974; Whitney and Krebs, 1975; Schaub and Larsen, 1978; Fellers, 1979a; Kupferberg, 1998). The main question then, becomes whether the frequency or severity of limb malformations has increased. In a comparison of recent (1990–2000) and historical (1899–1989) publications on the subject, we found that recent reports document (1) a wider range of severe malformations; (2) a greater number of affected amphibian species; (3) a larger number of affected sites; and (4) a higher frequency range of affected individuals at reported sites. These conclusions parallel those of Hoppe (2000; this volume), whose Minnesota study was conducted with a unique synthesis of museum voucher inspections and re-survey data.

What we cannot know for certain is the degree to which historical publications and museum specimens are representative of the historical condition. Substantial biases with respect to sampling methods, numbers of researchers, and the focus of historic and recent studies preclude a rigorous testing of our null hypothesis. Of interest, however, are the historic reports documenting large numbers of malformed amphibians. While similar to recent reports in many respects, it is notable that these accounts are few in number (n = 4), never describe more than one affected species from a site, and discuss no abnormalities more severe than two extra limbs. A re-survey of one of these sites recorded higher rates of abnormalities and more affected species than did the survey 40 years prior. Qualitatively then, sufficient evidence exists to suggest that *Ribeiroia*-induced deformities may have increased in the West, either in scale (number of affected sites), intensity (frequency and severity of malformations), or both.

Human Impacts on Trematodes

Throughout the recent investigation of amphibian malformations, the potential causes have been divided artificially into "natural" (e.g., parasites and predators) and "anthropogenic" (e.g., pesticides and elevated UV-B radiation) factors. Use of this grossly over-simplified classification scheme leads to two perspectives: (1) assuming amphibian deformities recently have become more common or severe, "natural" agents, such as parasitism, are unlikely to explain such changes; and (2) sites for which *Ribeiroia* infection has been identified as the likely cause represent no concern from a conservation standpoint. Both perspectives are false. In truth, parasite infection is a *biotic* agent, which may be influenced substantially by interactions with other factors, whether biotic or abiotic. Human activity, for example, has frequently had a tremendous impact on the virulence of trematode-induced disease. We review the understudied realm of human impacts on the distribution and abundance of trematode populations. Each of the discussed mechanisms is then evaluated with respect to *Ribeiroia* infection in western North America.

Non-Indigenous Parasites

As global transportation achieves greater speed and efficiency, the introductions of non-indigenous animals, plants, and their respective parasites have become increasingly problematic (Elton, 1958; Vitousek et al., 1996; Lodge et al., 1998). Introduced parasites that successfully establish may have devastating effects on ecosystems as well as the economy (Stewart, 1991; Amin and Minckley, 1996; Barton, 1997). Native fauna, having no evolutionary resistance to the invading parasite, frequently exhibit heightened pathology and mortality following infection relative to hosts that have co-evolved with the parasite (Dobson and May, 1986). The colonization of a trematode to a new region requires the presence of suitable host species to support the life cycle, and records of digenetic trematode introductions are less common than many other direct life cycle parasites (Table 20-3; Kennedy, 1993; Dubois et al., 1996; Font, 1997). However, considering our limited knowledge on the range of most trematodes as well as their complicated taxonomy, it is likely the frequency of intercontinental (and especially intracontinental) trematode introductions remain underestimated.

Two of the most well-known examples of introduced trematodes are *Schistosoma mansoni* and *Fasciola hepatica*, both of which may have serious consequences on society. *Schistosoma mansoni*, the human blood fluke, traveled from Africa to South America, Central America, and the Caribbean Islands via the slave trade (D.S. Brown, 1978). *Fasciola hepatica*, the ruminant liver fluke of Europe, has colonized North America, Australia, New Zealand, Hawaii, and Papua New Guinea with the transport of infected stock (Boray, 1978). Both parasites remain important problems, with cases of human schistosomiasis estimated at 200 million and livestock industry losses due to liver flukes at over U.S. $2 billion (Stauffer et al., 1997; Boray and Munro, 1998).

Considering that several of the earliest records of *R. ondatrae* are in birds from Oregon (Price, 1931), Washington (McNeil, 1948), and California (Dubois and Mahon, 1959), we think it unlikely that *Ribeiroia* represents a recently introduced trematode in the western United States. Moreover, *Ribeiroia* has probably caused amphibian limb deformities for at least 40 years and probably much longer. Johnson et al. (2002) recently found dense infections of *Ribeiroia* in severely deformed Pacific treefrogs from a pond in western Montana which, during the late 1950s, supported similarly abnormal treefrogs (Hebard and Brunson, 1963). We suspect that *R. ondatrae* infection explains most of the limb malformations in both accounts.

Although not exotic in western North America, *R. ondatrae* may have been aided greatly in its dispersal by the intentional and unintentional introductions of non-indigenous amphibians and game fish. Both groups have functioned as important vectors for trematode introduction in other parts of the world (Table 20-3) and have been disseminated widely within the western states. Introduced frog species currently established in western regions include Rio Grande leopard frogs *(R. berlandieri)*, American bullfrogs *(R. catesbeiana)*, green frogs *(R. clamitans)*, southern leopard frogs *(R. sphenocephala)*, and African-clawed frogs. Bullfrogs, in particular, have spread widely following their introduction at the turn of the century (Hayes and Jennings, 1986). The stocking of game fish, particularly salmonids and centrarchids, remains a widespread practice impacting a diverse array of lentic and lotic habitats. Greater than 50% of the freshwater fish in California are non-indigenous (Moyle, 1976; Vitousek et al., 1996).

TABLE 20-3
Introductions of Non-indigenous Trematodes

Species	*Subclass*	*Family*	*Native*	*Recently Colonized*	*Vector*
Amurotrema dombrowskaje	Digenea	Diplodiscidae	East Asia	Central Asia	exotic fish[a]
Diplodiscus megalodiscus	Digenea	Diplodiscidae	Australia	New Zealand	frog[f]
Ascocotyle tenuicollis	Digenea	Heterphyiidae	North America	Hawaii	exotic fish[b]
Brachylaima apoplania	Digenea	Brachylaimidae	Southeast Asia	New Zealand	polynesian rat[c]
Calicophoron calicophorum	Digenea	Paramphistomidae	Europe	Australia	ruminants[d]
Orthocoelium streptocoelium	Digenea	Paramphistomidae	Europe	Australia	ruminants[d]
Paramphistomum ichikawai	Digenea	Paramphistomidae	Europe	Australia	ruminants[d]
Cyathocotyle bushiensis	Digenea	Cyathocotylidae	North America	Canada	infected snail[e]
Fasciola hepatica	Digenea	Fasciolidae	Europe	Australia, North America, New Zealand, Hawaii, Papua New Guinea	ruminants[d,g]
Gorgodera australiensis	Digenea	Gorgoderidae	Australia	New Zealand	frog[f]
Lissorchis attenuatum	Digenea	Lissorchidae		Canada	exotic fish[h]
Mesocoelium monas	Digenea	Mesocoeliidae	South America	New Guinea	toad[f]
Notocotylus gippyensis	Digenea	Notocotylidae	New Zealand	Europe	infected snail[d]
Ornithodiplostomum ptychocheilus	Digenea	Diplostomatidae	North America	Arizona Reservoir	exotic fish[i]
Posthodiplostomum minimum	Digenea	Diplostomatidae	North America	Arizona Reservoir	exotic fish[i]
Sanguinocola inermis	Digenea	Sanguincolidae		Europe	[j]
Schistosoma mansoni	Digenea	Schistomatidae	Africa	South America, Caribbean	slave trade[k]
Cleidodiscus pricei	Monogenea	Dactylogyridae	North America	Europe	exotic fish[a]
Dactylogyrus extensus	Monogenea	Dactylogyridae	Europe	North America	exotic fish[a,j]
Dactylogyrus vastator	Monogenea	Dactylogyridae	Japan	North America, Europe	exotic fish[a,j]
Dactylogyrus anchoratus	Monogenea	Dactylogyridae	Japan	North America, Europe	exotic fish[a,j]
Dactylogyrus aristichthys	Monogenea	Dactylogyridae	East Asia	Central Asia, North America, Europe	exotic fish[l]
Dactylogyrus baueri	Monogenea	Dactylogyridae	Japan	North America	exotic fish[l]
Dactylogyrus chenshuchenae	Monogenea	Dactylogyridae	East Asia	Central Asia, North America, Europe	exotic fish[l]
Dactylogyrus ctenopharyngodonis	Monogenea	Dactylogyridae	East Asia	Central Asia, North America, Europe	exotic fish[l]
Dactylogyrus formosus	Monogenea	Dactylogyridae	Japan	North America	exotic fish[l]
Dactylogyrus hypothalmichthys	Monogenea	Dactylogyridae	East Asia	Central Asia, North America, Europe	exotic fish[l]
Dactylogyrus lamellatus	Monogenea	Dactylogyridae	East Asia	Central Asia, North America, Europe	exotic fish[l]
Dactylogyrus minutus	Monogenea	Dactylogyridae	Europe	North America	exotic fish[a]

TABLE 20-3 (continued)

Species	*Subclass*	*Family*	*Native*	*Recently Colonized*	*Vector*
Dactylogyrus skrjabini	Monogenea	Dactylogyridae	East Asia	Central Asia, North America, Europe	exotic fish[l]
Dactylogyrus suchengtai	Monogenea	Dactylogyridae	East Asia	Central Asia, North America, Europe	exotic fish[l]
Pseudodactylogyrus anguillae	Monogenea	Dactylogyridae		Europe	[j]
Pseudodactylogyrus bini	Monogenea	Dactylogyridae	East Asia	Europe	[j]
Gyrodactylus ctenopharyngodonis	Monogenea	Gyrodactylidae		Central Asia, North America, Europe	exotic fish[l]
Gyrodactylus cyprini	Monogenea	Gyrodactylidae	Europe	North America	exotic fish[a]
Gyrodactylus salaris	Monogenea	Gyrodactylidae	Sweden	Norway	exotic fish[a]
Nitzschia sturionis	Monogenea	Capsalidae	Caspian Sea	Aral Sea	exotic fish[a]
Urocleidus principalis	Monogenea	Ancyrocephalidae	North America	Europe	[j]
Urocleidus dispar	Monogenea	Ancyrocephalidae	North America	Europe	exotic fish[a]
Urocleidus furcatus	Monogenea	Ancyrocephalidae	North America	Europe	exotic fish[a]
Urocleidus similis	Monogenea	Ancyrocephalidae	North America	Europe	exotic fish[a]

[a]Bauer (1991), [b]Font (1997), [c]Wheeler et al. (1989), [d]Cribb (1990), [e]Dobson and May (1986), [f]Prudhoe and Bray (1982), [g]Boray (1978), [h]Dubois et al. (1996), [i]Amin and Minckley (1996), [j]Kennedy (1993), [k]Brown (1978), and [l]Bauer and Hoffman (1976).

NOTE: When available, the suspected transport vector is provided in the final column.

Non-indigenous Host Species

Many trematodes exhibit considerable specificity in the use of a first intermediate host, and it is often the distribution of the snail host that limits a parasite's range. Mammalian and avian definitive hosts are highly vagile and facilitate trematode dispersal, but establishment will only occur if the proper intermediate hosts are available (Esch et al., 1988). Unfortunately, human-mediated introductions and the subsequent establishment of freshwater mollusks, including many hosts of waterborne diseases (e.g., schistosomiasis, fascioliasis, and paragonimiasis), are frequent and may result in substantial financial (e.g., zebra mussel, *Dreissena polymorpha*) or ecological (e.g., Asian clam, *Corbicula fluminea*) repercussions (Table 20-4). As with trematodes, many more introductions of non-indigenous snails undoubtedly go unreported.

Under natural conditions, aquatic snails and their eggs are distributed passively on the plumage and feet of birds and, to a lesser extent, mammals and insects (Rees, 1965; Prentice, 1983; Boag, 1986). Human activity considerably increases the range and likelihood of such passive transport and acts primarily through the following four pathways:

1. Aquatic plant trade (Boray, 1978; Pointier et al., 1993; Madsen and Frandsen, 1989)
2. Aquarium fish (Woodruff et al., 1985; Bowler and Frest, 1992; Warren, 1997)
3. Aquaculture and fish farms (D.S. Brown, 1980)
4. Ballast water (Stewart, 1991; Carlton, 1992)

Many of these activities are only loosely regulated and, while snails are routinely intercepted by national inspectors (Hanna, 1966), it is clear that many escape detection.

Although less likely to be infected at the time of introduction, the establishment of non-native snails may drastically alter the epidemiology of endemic pathogens. The most notable examples come from the fasciolid trematodes or liver flukes. *Pseudocussinea columella*, a North American snail, has been translocated across the globe (Table 20-4) with important consequences on the transmission of *F. hepatica*. In both New Zealand and Australia, the semi-amphibious *P. columella* expanded into habitats historically uncolonized by native lymnaeids, leading to fascioliasis in previously unaffected regions (Boray, 1978; D.S. Brown, 1978). Similarly, the rapid colonization of a series of dams in South Africa by *P. columella* has increased the prevalence and intensity of infection in local ruminants, resulting in substantial financial loss (D.S. Brown, 1980).

The influence of introduced snails on the epidemiology of *Ribeiroia* is difficult to determine. All available data on *Ribeiroia* indicate that species in the genus require snails in the family Planorbidae as their first intermediate hosts (Beaver, 1939; Basch and Sturrock, 1969; Schmidt and Fried, 1997; Johnson et al., 2002). Planorbid snails, including a number of documented *Ribeiroia* host species, have been widely introduced in North America (Table 20-3) but their influence on the parasite's range has not been studied. Malek (1977) recorded *Ribeiroia* in *Biomphalaria obstructa*, a South American planorbid, at a site in Louisiana. Intracontinental transfers of snails may also be important, and Johnson et al. (2002) collected two planorbid species in the western United States that were beyond their known range. Populations of each species hosted *Ribeiroia* infections at several wetlands (Johnson et al., 2002). Another source of intracontinental transfers is aquarium stores. *Planorbella tenuis*, a known *Ribeiroia* host, and several other planorbid species have been routinely observed at southern California pet stores (Johnson and Lunde, personal observation). If released in the wild, these snails could establish at previously uncolonized wetlands or increase the local planorbid density at established sites, as has been recorded in other parts of the United States (Bowler and Frest, 1992) and South America (Correa et al., 1980). Finally, aquatic researchers themselves may act as unwitting vectors, transporting snails in their muddy boots and nets as they move among wetlands. This emphasizes the importance of rigorously sterilizing sampling equipment between sampling sites.

Habitat Modification—Snail Hosts

Human modification of aquatic systems can lead to increases of human and bovine diseases resulting from trematode infection, such as schistosomiasis, paragonimiasis, clonorchiasis, and fascioliasis (Madsen and Frandsen, 1989). Water impoundments (e.g., reservoirs and hydroelectric dams), often improve snail habitat and concentrate human populations, both of which facilitate schistosome transmission and have historically been a source of great misery in the tropics and neotropics (Jordan et al., 1980; Roberts and Janovy, 1996; Sutherland, this volume). Intermediate hosts of mesenteric and urinary schistosomiasis, planorbid snails of the genus *Biomphalaria* and *Bulinus*, respectively, rapidly colonize and establish dense populations in such habitats, leading to disease oubreaks as documented from Lakes Volta, Kariba, and Nasser in Africa (D.S. Brown, 1980; Jordan et al., 1980). More recently, Southgate (1997) reported a severe increase in both schistosome species following the construction of two dams in the Senegal River Basin. Prior to 1985, the diseases were virtually foreign to the populace, but within 5 years of the dams' completion many of the surrounding villages suffered human infection prevalences of 80% or greater.

This problem is not confined only to large dams. Irrigation schemes, borrow pits, quarries, drainage ditches, small impoundments, and aquaculture ponds have all been associated with elevated snail populations and trematode infection intensities, sometimes with serious economic impacts (Correa et al., 1980; D.S. Brown, 1980; Jordan et al., 1980; Woodruff et al., 1985; Ripert and Raccurt, 1987; Madsen and Frandsen, 1989; Bowler and Frest, 1992). Coates and Redding-Coates (1981), examining agricultural practices in Sudan, identified the increased snail densities associated with irrigation canals as responsible for the high prevalence of schistosomes and the resultant financial loss. Similar irrigaton systems outside of western Australia have recently been colonized by the lymnaeid snail host of *F. hepatica;* in Rhodesia the construction of numerous small dams was blamed for the subsequent increase in *Fasciola* infection and the consequent condemnation of bovine liver meat (D.S. Brown, 1980; Boray and Munro, 1998).

In the western United States, as in other parts of the world, aquatic systems have been altered extensively by human activity. In geologically active California, where natural lakes and ponds are rare, nearly every river and stream with a large and constant flow has been dammed, frequently facilitating the establishment of exotic species (Moyle, 1976; McGinnis, 1984; Schoenherr, 1992). One of the most common impoundments is the farm pond, approximately 80% of which were built

TABLE 20-4
The Global Translocations of Freshwater Snails (Pulmonates and Prosobranchs)

Species	*Family*	*Native*	*Recently Colonized*
Biomphalaria straminea	Planorbidae	South America	China, Australia, Caribbean, Central America[a,b,c]
Biomphalaria glabrata	Planorbidae	South America, West Indies	North America[d,e]
Biomphalaria havanesis	Planorbidae	Cuba, West Indies	North America[e]
Biomphalaria obstructa	Planorbidae	Mexico, South America	North America[f,g,h]
Planorbella duryi	Planorbidae	North America (Florida)	Europe, Africa, Middle East, South America, (Western) North America[a,f,i]
Helisoma anceps	Planorbidae	North America	Europe[j]
Planorbis corneus	Planorbidae	Europe	Australia[k]
Menetus dilatatus	Planorbidae	North America	Europe[l]
Indoplanorbis exustus	Planorbidae	India, Southeast Asia	Africa, Australia[a]
Drepanotrema aeruginosus	Planorbidae	Central America	North America[d]
Drepanotrema cimex	Planorbidae	Central America	North America[d]
Drepanotrema kermatoides	Planorbidae	Central America	North America[d]
Pseudosuccinea columella	Lymnaeidae	(Eastern) North America	Australia, New Zealand, Africa, South America, Caribbean, Europe, (Western) North America, Hawaii[a,f,k,m,n]
Lymnaea viridis	Lymnaeidae	Papua New Guinea, Guam	Australia, China, Japan, Philippines[k,m]
Lymnaea auricularia rubiginosa	Lymnaeidae	India	Australia[a,k,m]
Lymnaea peregra	Lymnaeidae	Europe	Australia[m]
Lymnaea stagnalis	Lymnaeidae	Europe	New Zealand, Australia[k]
Fossaria truncatula	Lymnaeidae	Europe	New Zealand[m]
Radix auricularia	Lymnaeidae	Europe, Asia	North America[d,e,f,o,p]
Lymnaea natalensis	Lymnaeidae	South Africa	Denmark[a]
Physella acuta	Physidae	North America	Europe, Africa, Australia, Middle East, Far East[a,d,n]
Physa waterloti	Physidae		Denmark[a]
Stenophysa marmorata	Physidae	South America, West Indies	North America[d]
Stenophysa maugeriae	Physidae	Mexico	North America[d]
Thiara granifera	Thiaridae	Far East	North America, South America, Caribbean[a,d,q,r,s]
Thiara granifera mauiensis	Thiaridae	Hawaii	California[f]
Tarebia tuberculata	Thiaridae	South America, Asia, Africa, India, East Indies	North America, Europe, Caribbean[a,d,f,s]
Melanoides turriculus	Thiaridae	Asia, Africa	North America[d,s]
Bithynia tentaculata	Bithyniidae	Europe	North America[d,f,q]
Marisa cornuarietis	Ampullaridae	South America	North America, Caribbean[d,s,t,u]
Pomacea bridgesi	Ampullaridae	South America	North America[d,s]
Pomacea canaliculata	Ampullaridae	South America	North America, Far East, Southeast Asia, Philippines[d,v]
Pomacea haustrum	Ampullaridae	South America	North America[d]
Potamopyrgus antipodarum	Hydrobiidae	New Zealand	Europe, North America, Australia, Corsica[d,e,k,t,w]
Cipangopaludina chinensis malleata	Viviparidae	Japan	North America[d,f]

TABLE 20-4 (continued)

Species	*Family*	*Native*	*Recently Colonized*
Cipangopaludina japonica	Viviparidae	Japan	North America[d]
Elimia livescens livescens	Pleuroceridae	Great Lakes to S. Quebec, Indiana, Illinois, Ohio	Hudson River Drainage[e]
Elimia virginica	Pleuroceridae	Massachusetts to Virginia	Great Lakes Basin[e]
Valvata piscinalis	Valvatidae	Europe	North America[q]

[a]Madsen and Frandsen (1989), [b]Pointier et al. (1993), [c]Woodruff et al. (1985), [d]Turgeon et al. (1998), [e]Bowler and Frest (1992), [f]Taylor (1981), [g]U.S. Congress (1993), [h]Malek (1977), [i]Roushdy and El-Eniani (1981), [j]Henrard (1968), [k]Walker (1998), [l]Boycott (1936), [m]Boray (1978), [n]D.S. Brown (1967), [o]Metcalf and Smartt (1972), [p]Hanna (1966), [q]Abbott (1950), [r]Prentice (1983), [s]Warren (1997), [t]Brown (1980), [u]Burch (1989), [v]Vitousek et al. (1996), and [w]Cribb (1990).

to support stock grazing (Bennett, 1971). Since the 1930s, extensive pond building by organizations such as the Soil Conservation Service has resulted in an increase of artificial wetlands in the United States of 2.9 million acres (Bennett, 1971; Tiner, 1984; Dahl and Johnson, 1991; Leja, 1998). California's Central Valley has gained 27.9 thousand acres since 1939—a 300% increase (Frayer et al., 1989). Unfortunately, little is known about the ecological importance of such habitats. Some native amphibians clearly utilize farm ponds if the non-indigenous species load (i.e., introduced fishes and American bullfrogs) is not extreme (Fisher and Shaffer, 1996; Hayes and Jennings, 1989; Stebbins, 1985). Similar ponds in England provide important snail habitats (Boycott, 1936). Considering the abiotic and biotic profiles of such ponds, we suspect these anthropogenic habitats, in contrast to larger reservoirs, are particularly conducive to *Ribeiroia* parasites and amphibian limb malformations, primarily through their effects on snail hosts.

One important feature of farm ponds is their productivity. Frequently a source of water for cattle and agricultural irrigation, these ponds are often eutrophic. Productivity is considered an important determinant of snail distributions, and mildly eutrophic water systems are associated with greater snail diversity and total gastropod biomass (Carr and Hiltunen, 1965; Harman and Forney, 1970; Russell-Hunter, 1978). What little experimental work has been done suggests that, in more productive environments, aquatic snails (1) grow faster, (2) reach a larger maximum size, (3) are more fecund, and (4) achieve greater densities (Eversole, 1978; Brown and DeVries, 1985; K.M. Brown et al., 1988). Helisome snails, the first intermediate hosts for trematodes in the genus *Ribeiroia*, may be particularly favored in nutrient-enriched environments (but see Bovbjerg and Ulmer, 1960; Bovbjerg, 1980). These snails are commonly found in permanent, eutrophic, often manmade bodies of water (Boycott, 1936; Boerger, 1975; Eversole, 1978; Taylor, 1981; K.M. Brown, 1982; Fernandez and Esch, 1991). Chase (1998) found a positive relationship between productivity and the relative biomass of the snail *H. trivolvis* among 29 temperate ponds in Michigan, suggesting that these snails are more successful in productive environments. According to Chase (1998), elevated productivity can have important mediating effects on the predation of helisomes in permanent ponds, where predation is an important regulator of snail species composition and abundance (Brown and DeVries, 1985; Lodge et al., 1987; K.M. Brown, 1991). In a series of mesocosm and *in situ* field experiments, Chase (1998) determined that more productive environments favored helisome snails by facilitating faster growth, thus allowing them to achieve size refugia from predators and a greater total biomass in the long term.

Taken together, these studies suggest that eutrophic ponds will support greater densities of helisome snails which, depending on definitive host activity, could lead to an increased number of *Ribeiroia*-infected snails. More infected snails, in turn, would lead to higher intensities of infection in larval amphibians. According to the results of Johnson et al. (1999, 2001a), heavier *Ribeiroia* infections cause higher rates of more severe limb deformities in metamorphic anurans, suggesting such ponds might have important consequences on the local frequency of malformations.

Although eutrophication is a natural event, humans frequently accelerate the process via increased nitrogen and phosphorus inputs from fertilizer run-off, cattle defecation, and domestic sewage. This "cultural" eutrophication is an increasing problem in aquatic systems, where it often occurs more rapidly than the ecosystem can adapt (Valtonen et al., 1997). If extreme, the result is a community with low species diversity and high densities of a few, over-represented species (Dobson and May, 1986). In the course of our own fieldwork, we observed substantial differences in the *Ribeiroia* host-parasite complex between culturally eutrophied ponds (e.g., Jette Pond in Montana; Fig. 20-4A) and naturally eutrophic ponds (e.g., Lassen Volcanic National Park in California; Fig. 20-4B). With a 100-year intensive grazing history, Jette Pond is highly eutrophic with an abnormality frequency of 50% and a dense helisome population. Several ponds at Lassen Park, in contrast, exhibit low abnormality frequencies (4–8%) and support small snail populations (Johnson et al., 2002). Although more field and experimental research is necessary to test a hypothesis causally connecting increasing productivity and amphibian abnormalities, eutrophication is already an implicated cause in helminth outbreaks from other parts of the world (D.S. Brown, 1980; Northcote, 1992; de Gentile et al., 1996; Bohl, 1997). This relationship should not, however, be expected in all eutrophic habitats. Hypereutrophic ponds, in which the oxygen levels become depleted from the excess primary productivity, or ponds contaminated by pesticides associated with agricultural habitats, are likely to prove detrimental to snails and amphibians alike (Harman, 1974; Leja, 1998).

Habitat Modification—Avian Hosts

Human modifications to the aquatic environment have also changed the activity and population sizes of many waterbirds,

some of which serve as definitive hosts for *Ribeiroia*. By extension, these alterations affect the distribution and abundance of avian trematode communities. Aquatic habitat modifications could contribute to the apparent increase of amphibian malformations by either (1) increasing the number of water bodies supporting the *Ribeiroia* life cycle or (2) increasing the intensity of *Ribeiroia* at sites already supporting the parasite. Through their impacts on bird populations, the following trends support one or both of the above mechanisms: disappearing natural wetlands, growing numbers of artificial aquatic habitats, and increasing bird populations.

Since colonial times, the United States has developed an estimated 117 million acres of natural wetlands, with more than 4.5 million acres lost in California alone—a 91% decrease (Dahl, 1990). The destruction of natural wetlands displaces definitive hosts into alternate wetlands, causing birds to concentrate in remaining wetlands (Banks and Springer, 1994; Krapu, 1996). Higher densities of birds at these sites can lead to higher trematode infection rates of intermediate snail hosts (Bustnes and Galaktionov, 1999) and thus higher infection intensities in second intermediate hosts. In the case of *Ribeiroia*, this could directly increase both the frequency and severity of amphibian limb malformations (Johnson et al., 1999). Indeed, increased densities of waterfowl at several European lakes have been associated with outbreaks of swimmers itch, a skin condition caused by avian trematodes (de Gentile et al., 1996).

The colonization of manmade wetlands by definitive hosts can broaden the distribution of the host's trematode community. As birds are perpetually forced out of disappearing natural habitats, they are becoming increasingly dependent on the growing number of artificial wetlands (Ruwaldt et al., 1979; Hudson, 1983). These alternative sites are not always suboptimal; adaptable species may even thrive in such conditions. Destruction of coastal wetlands have displaced lesser snow geese (form of *Chen caerulescens*) to inland rice fields, where overwintering populations in Texas have nearly doubled in 30 years (Robertson and Slack, 1995). Cattle and farm ponds built from the 1950s to the 1980s comprised the majority of the 2.7-million-acre increase in palustrine, non-vegetated wetlands in the United States (Tiner, 1984; Dahl and Johnson, 1991). These abundant aquatic habitats provide important colonization opportunities for wildlife. They may also be eutrophic, and, as previously discussed, are therefore more likely to support dense helisome populations—the first intermediate hosts of *Ribeiroia*. Artificial ponds, reservoirs, and fish farms also attract definitive hosts of *Ribeiroia* such as herons, egrets, and mallards (Ruwaldt et al., 1979; Bildstein et al., 1994; Glahn et al., 1999). By increasing the likelihood that appropriate hosts will converge in aquatic habitats, these anthropogenic water bodies provide opportunities for the *Ribeiroia* life cycle to establish, possibly contributing to the apparent increase in the number of wetlands with deformed amphibians. However, by its very dependence on artifical (as opposed to natural) wetlands, this pattern also brings malformations in closer contact with human populations where they are more likely to be observed and reported.

Birds that can adapt to, or even take advantage of, massive alterations in their environment may expand their populations, as reported for certain *Ribeiroia* hosts (Table 20-5). Great blue heron populations have increased nationwide by 47% in the last 30 years and are growing substantially in California, Oregon, and Washington ($p < 0.05$; Price et al., 1995; Sauer et al., 1996). As a group, wading birds are highly adaptable to human-modified environments such as rice fields, fish farms, reservoirs, farm ponds, and wastewater treatment ponds (Hoy, 1994; Stickley et al., 1995; Day and Colwell, 1998; Elphick and Oring, 1998). The flexible foraging habitats of great blue herons, along with their broad diet (e.g., fish, frogs, insects, and small mammals) are likely contributors to their population expansions (Butler, 1992). Increases in the population size of *Ribeiroia* definitive hosts will either concentrate birds at existing wetlands or encourage the colonization of new wetlands. Either of these outcomes could effectively increase the prevalence and/or severity of amphibian malformations, depending on the abundance of *Ribeiroia* and its intermediate hosts (helisome snails and amphibians). Unfortunately, we know little about the definitive hosts of *Ribeiroia* in the western United States, and the importance of different waterbird species to the transmission of this parasite requires further investigation.

Conclusions and Continued Study

Increasing reports of malformed amphibians from across North America have generated substantial concern over the implications of the phenomenon for environmental health. In the western United States, amphibian malformations are frequently associated with infection by a trematode in the genus *Ribeiroia*. This paper evaluates the importance of *Ribeiroia* infection and the resulting pathology of limb malformations from a conservation standpoint—do they represent a concern?

Trematode parasites, of which more than 150 species utilize amphibians as intermediate hosts, are infrequently studied with respect to their impacts on amphibian distributions and populations dynamics (but see Kiesecker and Skelly, 2000, 2001). Field and laboratory studies of *Ribeiroia* infection suggest this trematode may substantially reduce amphibian survivorship through two mechanisms: direct mortality due to infection and indirect mortality resulting from impaired fitness associated with limb malformations. Experimental exposures of larval Pacific treefrogs to cercariae resulted in nearly 50% direct mortality, and malformed metamorphic frogs in the wild are not expected to survive to sexual maturity (Johnson et al., 1999). The long-term effects of infection on a population are not yet known, but *Ribeiroia* has recently been recorded in a number of amphibian species of concern status in the West (Table 20-1). While it is unlikely the parasite is the major factor behind these declines, amphibian deformities may represent an added insult to already precarious populations, particularly if such deformities are increasing in frequency.

The conservation importance of *Ribeiroia* infection and limb malformations largely depends on whether the rate or prevalence of the induced abnormalities has increased in recent years. If the current rate of parasite-induced deformities is equivalent to the historical baseline, we would expect that native amphibians have adapted to accommodate them or previously gone extinct. If however, *Ribeiroia* is a recent invader or has increased in intensity or range, these deformities may be cause for concern. On the basis of comparisons between recent and historic literature on amphibian abnormalities, there is evidence suggestive of an increase in the severity and distribution of limb malformations. At this time, questions regarding the representative nature of the historic data prevent a rigorous, quantitative test of this hypothesis. However, reports over the last decade do document higher rates of more severe malformations from a larger number of sites and species than noted in the historical precedent. Within a limited examination of amphibian vouchers from two museum collections, abnormalities were infrequent and typically minor, and records

TABLE 20-5
Population Trends in Avian *Ribeiroia* Hosts in North America

Species	*United States Population Change*[a]	*United States Population Change*[b]	*States with Substantial Increase in the West*[c]	*Source*
Common loon *(Gavia immer)*	+	N/A	N/A	Kinsella and Forrester (1999); (1994 Conboy USNPC)[d]
Brown pelican *(Pelecanus occidentalis)*	0	+		(1994 Roderick USNPC)[d]
Olivaceus cormorant *(Phalacrocorax olivaceus)*	0	+		Ramos Ramos (1995)
Great blue heron *(Ardea herodias)*	+	+	CA, OR, WA	M. Kinsella, unpublished data
Great egret *(Ardea albus)*	0	+	CA	Travassos (1939) as cited by Cable et al. (1960); Soledad Sepulveda et al. (1999); HWML[e]
Little blue heron *(Egretta caerulea)*	0	0		Cable et al. (1960); Basch and Sturrock (1969); Soledad Sepulveda et al. (1996)
Tricolored heron *(Egretta tricolor)*	0	0		Lumsden and Zishke (1963)
Green heron *(Butorides virescens)*	0	+		Riggin (1956)
Black-crowned night-heron *(Nycticorax nyticorax)*	0	+	CA, OR	Pineda et al. (1985)
Reddish egret *(Egretta rufescens)*	0	0		(1977 Paul USNPC)[d]
Wood duck *(Aix sponsa)*	+	0	OR, WA	Thul et al. (1985)
Mallard *(Anas platyrhynchos)*	0	−	CA	(1993 Fedynich USNPC)[d]; HWML[e]
Common merganser *(Mergus merganser)*	+	0	CA, OR	(1947 Zimmerman USNPC)[d]
Red-breasted merganser *(Mergus serrator)*	0	0		(1932 Woodbury USNPC)[d]
Osprey *(Pandion haliaetus)*	+	+	CA	Beaver (1939); Taft et al. (1993); Kinsella et al. (1996)
Bald eagle *(Haliaeetus leucocephalus)*	+	0	OR, WA, ID, MT	Tuggle and Schmeling (1982)
Cooper's hawk *(Accipiter cooperii)*	+	0	CA, WA, ID	Beaver (1939)
Broad-winged hawk *(Buteo platypterus)*	0	0		Taft et al. (1993)
Red-tailed hawk *(Buteo jamaicensis)*	+	+	CA, OR, WA, ID	Taft et al. (1993)
California gull *(Larus californicus)*	0	N/A	N/A	Price (1931)
Great horned owl *(Bubo virginianus)*	0	+	CA, OR, WA	Taft et al. (1993); HWML[e]

[a] Population trends (1966–93) from Price et al. (1995). Significant change when $p < 0.05$; 0 = no significant change.

[b] Population trends (1959–88) from Sauer et al. (1996). Significant change when $p < 0.05$; 0 = no significant change.

[c] Western States include California, Oregon, Washington, Idaho, and Montana. Population trends from Sauer et al. (1996). Significant change when $p < 0.05$.

[d] United States National Parasite Collection at http://www.lpsi.barc.usda.gov/BNPCU/parasrch.htm

[e] Harold W. Manter Laboratory, Division of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska

NOTE: N/A designates when data were not available.

of *Ribeiroia* infection from wild amphibians are non-existent prior to 1999.

Over the last 300 years, humans have altered dramatically the distribution of plants, animals, and their respective parasites. In reviewing some of this literature, it is immediately apparent that human activity frequently favors trematode populations, sometimes with costly repercussions. The interactions between human disturbance and wildlife diseases are exceedingly complex and may assume direct, indirect, or synergistic mechanisms (Möller, 1987; Khan and Thulin, 1991). Based on existing evidence, *Ribeiroia* is not a recently introduced species nor has its distribution been dramatically affected by the colonization of a non-indigenous host species. An interaction between pesticide contamination and elevated parasite infection is also not suspected, given the rarity of pesticide presence at amphibian malformation field sites in the western United States. If *Ribeiroia* has increased or shifted its range within the western United States, we suspect it is a result of extensive

human alterations to aquatic systems. The life cycle of *Ribeiroia* (Fig. 20-2), which depends on helisome snails, amphibian larvae, and waterbirds, appears to thrive in artificial impoundments. More specifically, highly productive farm ponds can support dense helisome snail populations. Widespread construction of these understudied habitats may have facilitated large range shifts of helisome snails and, consequently, of their trematodes. The accelerated, cultural eutrophication of farm ponds, due to cattle and agricultural fertilizers, create the appropriate conditions for elevated snail densities, possibly increasing *Ribeiroia* infection intensities in amphibians from mild, nonpathological levels to extremely heavy infections, with the resulting malformations threatening the longevity of amphibian host populations. There is also evidence that, as natural wetlands are continually altered or destroyed, these anthropogenic habitats become increasingly important foraging areas for waterbirds, including several known *Ribeiroia* definitive hosts.

At this point, our hypotheses, while well founded in theory and precedents, remain speculative. The study of *Ribeiroia*, its ecology, and unique pathology in amphibians is still relatively new. In the continued research of amphibian limb malformations in the West and the *Ribeiroia* host-parasite complex, we emphasize the following areas of study: (1) broad-based field surveys comparing parasite dynamics (e.g., host densities, infection intensities, and abnormality rates) between natural and human-modified systems; (2) *in situ* experimental studies on the effects of increasing productivity on helisome density and *Ribeiroia* infection prevalence; (3) waterbird helminth surveys to determine the definitive hosts of *Ribeiroia* in the West; and (4) long-term monitoring on the impacts of *Ribeiroia* infection and limb deformities on amphibians, particularly for those species and populations currently in decline.

Summary

Numerous reports of malformed amphibians from across North America have prompted investigations into the cause(s) of the abnormalities and their implications for affected populations. While many agents may be responsible for the national phenomenon, reports of amphibian limb malformations in the western United States correlate strongly with infection by a parasitic trematode *(Ribeiroia ondatrae)*. In laboratory experiments *Ribeiroia* causes high frequencies of limb malformations and reduced survivorship in metamorphosing anurans. Although similar malformations have been recorded in the West over the last century, reports from the last decade detail a greater frequency range of more severe malformations from a larger number of sites and species. The intensity of trematode infections in other parts of the world has been dramatically influenced by human activity, particularly via the transport of non-indigenous host species and the modification of wetland habitats. These changes have sometimes caused outbreaks of trematode-related diseases in intermediate host populations. Although not an exotic genus in the western United States, *Ribeiroia* may have increased recently in range or density in response to substantial alterations of western aquatic systems. Planorbid snails, the first intermediate hosts in the *Ribeiroia* life cycle, thrive in the permanent, highly productive ponds that have become an increasingly common habitat over the last 70 years. Cultural eutrophication, a frequent consequence of human activity around wetlands, can facilitate elevated densities of these snails and their resident parasites. Higher intensities of *Ribeiroia* infection translate directly to higher rates of more severe malformations in amphibian hosts. Additionally, human-modified wetlands serve as important foraging grounds for waterfowl, including many known *Ribeiroia* hosts. Increases in some of these species' populations have recently been recorded in the western United States.

Considering the debilitating nature of the observed limb malformations, their widespread occurrence in the West, and the number of amphibian species affected, we believe parasite-induced malformations may represent a concern deserving further investigation. In the continued study of this issue we advocate (1) an evaluation of the long-term, population-level threat to amphibians presented by *Ribeiroia* infection and the resulting malformations, and (2) field and laboratory studies on the effects of human activity, particularly elevated nutrient levels, on the *Ribeiroia* host-parasite complex and its pathology in amphibians.