Limb Deformities as an Emerging Parasitic Disease in Amphibians: Evidence from Museum Specimens and Resurvey Data

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Abstract: *Widespread reports of malformed amphibians are of growing conservation concern. Although accounts of mass malformations (>5%) in North American amphibian populations date back to the 1940s, they are often poorly documented and are rarely explained. We reviewed available information for nine historical accounts from California, Colorado, Idaho, Mississippi, Montana, Ohio, and Texas reported between 1946 and 1988. We then asked the following questions: (1) Which of these cases were associated with* Ribeiroia *(Trematoda: Digenea) infection? (2) Are malformations still occurring at these sites? And (3) if so, have the frequency or types of abnormalities changed? Each site was resurveyed between 1999 and 2002, and original voucher specimens were redescribed and examined for trematode infection. Direct identification and classification by discriminant function analysis indicated that historical malformations at six of eight sites were associated with infection by* Ribeiroia*, dating back as far as 1946. Malformations recorded historically at these sites were consistent with the documented effects of* Ribeiroia *infection, including extra limbs, cutaneous fusion, and bony triangles. Of the six sites that still supported amphibians upon resurvey, three continued to support severe limb malformations at frequencies of 7–50% in one or more species. Although no pesticides were detected, amphibians from each of these sites were infected with* Ribeiroia *metacercariae. Taken together, these results suggest that* Ribeiroia *infection has historically been an important cause of mass malformations in amphibians. We conclude that although parasite-induced malformations are not a new phenomenon, there is qualitative evidence suggesting that their prevalence has increased recently, and we highlight the need for long-term research to evaluate the impacts of malformations on amphibian population viability.*

Key Words: amphibian decline, amphibian deformities, emerging disease, malformations, museum study, parasites, *Riberiroia*, trematode

Extremidades Deformes como una Enfermedad Parasítica Emergente en Anfibios: Evidencia de Especimenes de Museo y Datos de Campo Nuevos

Resumen: *Los informes generalizados de anfibios malformados son motivo de creciente preocupación para la conservación. Aunque los registros de malformaciones masivas (>5%) en poblaciones de anfibios de Norte América datan de 1940, a menudo están escasamente documentados y rara vez explicados. Revisamos la información disponible para nueve informes históricos de California, Colorado, Idaho, Mississippi, Montana, Ohio y Texas entre 1946 y 1988. Posteriormente preguntamos: (1) ¿Cuáles de estos casos estaban asociados con la infección de* Ribeiroia *(Trematoda: Digenea)? (2) ¿Aún ocurren malformaciones en estos sitios? y (3)*

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Si es así, ¿ha cambiado la frecuencia o tipos de anormalidades? Cada sitio fue muestreado de nuevo entre 1999 y 2002 y los especimenes originales fueron descritos y examinados nuevamente para ver si tenían infección de trematodos. La identificación directa y la clasificación por análisis discriminante indicaron que las malformaciones históricas en seis de los ocho sitios estaban asociadas con infección por Ribeiroia*, que data desde 1946. Las malformaciones registradas históricamente en estos sitios fueron consistentes con los efectos documentados de infección de* Ribeiroia*, incluyendo extremidades adicionales, fusión cutánea y triángulos óseos. De los seis sitios donde aún había anfibios en los muestreos recientes, tres todavía presentaban malformaciones severas de las extremidades con frecuencias del 7 al 50% en una o más especies. Aunque no se detectaron pesticidas, los anfibios de cada uno de estos sitios estaban infectados con metacercarias de* Ribeiroia*. En conjunto, estos resultados sugieren que la infección por* Ribeiroia *históricamente ha sido una causa importante de malformaciones masivas en anfibios. Concluimos que, aunque las malformaciones inducidas por parásitos no son un fenómeno nuevo, existe evidencia cualitativa que sugiere que su prevalencia ha incrementado recientemente, y destacamos la necesidad de investigación a largo plazo para evaluar los impactos de malformaciones sobre la viabilidad de poblaciones de anfibios.*

Palabras Clave: declinación de anfibios, deformaciones en anfibios, enfermedad emergente, estudio museológico, malformaciones, parásitos, *Ribeiroia*, trematodo

Introduction

Two fundamental objectives of conservation science are to differentiate anthropogenic impacts on ecosystems from underlying natural variability and to identify the interface at which they interact. This process is complicated by indirect effects, synergistic interactions, and an inadequate knowledge of historical ecological conditions. Recently, widespread reports of amphibians with severe limb malformations have prompted concern over the possibility of an emerging conservation problem (Ouellet et al. 1997; Wake 1998; Burkhart et al. 2000; Blaustein & Johnson 2003). These malformations can affect a large percentage (>25%) of emerging frogs in a population and are suspected to impair chances of survival (e.g., Sessions & Ruth 1990; Johnson et al. 2001*b*). Malformations have accompanied mass die-offs in several anuran populations at one locality (Hoppe 2004). However, whether or not such malformations are indicative of environmental degradation or natural processes remains enigmatic, frustrating attempts to interpret the ecological implications of the phenomenon.

Malformed amphibians are not new; scattered reports of isolated frogs or toads with extra, missing, or abnormal limbs date back over 200 years (Ouellet 2000). J. Bland-Sutton noted in 1890, "To judge from published cases it would seem that supernumerary limbs in amphibia are uncommon, but inquiry satisfies me that they are, in frogs and toads at least, by no means infrequent." Less common, however, are documented occurrences involving high frequencies of malformations in a single population ("mass malformations"), as described in recent reports. Notable examples of such mass malformations were reported in Asia by Woitkewitsch (1959; Voitkevitch 1965), who examined nearly 500 *Rana ridibunda* with malformations ranging from completely missing limbs to eight supernumerary appendages, and in Europe by Henle (1981), who reported high levels (50%) of malformations in *Bufo viridis*, including extra limbs, jaw malformations, edemas, and limb hypoplasia. There also are historical examples of mass malformations in North American amphibians (Table 1), but the details are often scattered or incomplete. How these historical (pre-1990) accounts compare with recent reports of malformed amphibians, in terms of frequency, severity, and causative agent, has not been investigated. Whether these historical sites continue to produce malformed amphibians is also unknown. An in-depth review and comparison of such historical accounts in conjunction with examinations of any present-day deformities may help determine whether malformations observed recently represent a unique phenomenon.

A substantial obstacle to evaluating this question has been the lack of historical data on the frequency and severity of malformations. Evidence for a recent increase in the prevalence of abnormal amphibians is predominantly anecdotal, and there are no documented sites that, after years of supporting normal amphibians, have recently begun producing high frequencies of deformed animals. Instead, many "hotspot" sites were discovered only after the issue was publicized widely. In the absence of extant baseline data, examinations of vouchered museum specimens and resurveys of historical sites are important tools for evaluating environmental change. For example, Hoppe (2000), employing a combination of museum studies and field resurveys, found that the background rate of malformations in Minnesota leopard frogs (*Rana pipiens*) had increased from 0.4% in 1958–1963 to 2.5% in 1996–1997.

Recent findings have linked high frequencies of limb deformities to infection with a trematode parasite (*Ribeiroia ondatrae*; hereafter *Ribeiroia*) (Johnson

Table 1. Historical accounts (and resurvey sites) of mass malformations in amphibians from North American wetlands.

Year(s)	*Site and reference*[a]	*State*	*Coordinates*	*Elevation (m)*	*Species*	*Types*	*Suspected cause*[b]
1946–1947	Muskee Lake, 1–2	Nederland, Colorado	039°58′38″N 105°30′31″W	2520	*Ambystoma tigrinum*	hind polydactyly	genetic
1950–1951	Muskee Lake, 3	Nederland, Colorado	039°58′38″N 105°30′31″W	2520	*Ambystoma tigrinum*	hind polydactyly	environmental
1950–1951	Muskee Lake, 3	Nederland, Colorado	039°58′38″N 105°30′31″W	2520	*Rana pipiens*	hind polydactyly, "deformed limb"	environmental
1954–1955	Ripley Pond, 4–5	Ripley, Ohio	038°46′26″N 083°45′48″W	258	*Rana catesbeiana*	hind polymelia	chemical or genetic
1958	Phillips Bayou, 6–9	Tunica, Mississippi	034°32′42″N 090°30′28″W	55	*Rana catesbeiana*	hind polymelia	pesticides
1958–1961	Jette Pond, 10	Polson, Montana	047°47′27″N 114°15′27″W	2124	*Hyla regilla*	hind polymelia	injury or cattle
1964	Jette Pond, 11	Polson, Montana	047°47′27″N 114°15′27″W	2124	*Hyla regilla*	hind polymelia	injury or cattle
1974–1975	Jette Pond, 12	Polson, Montana	047°47′27″N 114°15′27″W	2124	*Hyla regilla*	hind polymelia	radiation
1961	Morgan Pond, 13	Franklin, Texas	030°58′34″N 096°24′50″W	130	*Rana catesbeiana*	hind polymelia	
1974	Dersch Meadows, 14	Lasser Volcano National Park, California	040°30′18″N 121°26′03″W	2102	*Hyla regilla*	hind polymelia	
1981	Suburban Pond, 15	Boise, Idaho	043°31′59″N 116°18′41″W	842	*Hyla regilla*	hind polymelia, polydactyly	insecticides
1986–1987	Seascape Pond, 16	Aptos, California	036°57′31″N 121°52′11″W	75	*Hyla regilla*	hind polymelia, polydactyly	trematodes
1986–1987	Seascape Pond, 16	Aptos, California	036°57′31″N 121°52′11″W	75	*Ambystoma macrodactylum croceum*	hind and fore polymelia, polydactyly	trematodes
1988	Veterans' Park, 17	Boise, Idaho	043°38′13″N 116°14′16″W	806	*Hyla regilla*	hind polymelia	insecticides

[a] *References: 1, Bishop & Hamilton 1947; 2, Bishop 1947; 3, Rosine 1955; 4, Anonymous 1954; 5, Hauver 1958; 6, Duncan 1958; 7, Pearson 1960; 8, Volpe 1977, 2000, personal communication; 9, Baker 1966; 10, Hebard & Brunson 1963; 11, R. Brunson 1999, personal communication, USNM 334545; 12, Anderson 1977, 1999, personal communication; 13, Anonymous 1962; 14, D. Lazaroff 1999, personal communication; 15, Reynolds & Stephens 1984; 16, Sessions & Ruth 1990; 17, T. Stephens 1999, personal communication and specimens.*

[b] *Original author(s) suspicions regarding the agent responsible.*

et al. 1999; Sessions et al. 1999). Across more than 60 wetlands in five western states (U.S.A.), *Ribeiroia* infection was significantly associated with above-baseline frequencies (>5%) of limb malformations in six amphibian species (Johnson et al. 2002). Although a low frequency of morphological anomalies can be expected in any population, this baseline frequency generally ranges between 0 and 5% in amphibian populations (e.g., Dubois 1979; Meyer-Rochow & Asashima 1988; Tyler 1998). In laboratory exposures, *Ribeiroia* causes high frequencies (40–100%) of malformations, including missing, extra, and deformed fore- and hind limbs. Amphibians exposed to a second trematode species or to no parasites exhibited significantly higher survivorship and normal development (Johnson et al. 1999, 2001*a*; Stopper et al. 2002). Field experiments further corroborate the link between *Ribeiroia* infection and amphibian malformations (Kiesecker 2002). However, whether these parasite-induced malformations represent a new or expanding phenomenon in wetland habitats remains conjectural. Prior to 1999, there were no records of *Ribeiroia* from wild-caught amphibians (Johnson & Lunde 2004).

Defined medically, a *disease* represents any deviation from or impairment of the normal structure or function of any part, organ, or system of the living animal or plant body (Merriam-Webster 1997; U.S. Food and Drug Administration 1999). Clinical signs of a disease may be morphological, physiological, or behavioral, and the etiologic agent may be infectious, genetic, nutritional, toxicological, or traumatic. Here, we classify parasite-induced malformations in amphibians as a disease, recognizing the gross morphological alterations and elevated mortality resulting, directly or indirectly, from infection. Other pathogens, such as mousepox virus, (ectromelia) for example, are also known to induce limb abnormalities within infected hosts (Greenwood et al. 1936).

Our objectives were to (1) investigate the role of *Ribeiroia* infection in historical accounts of mass malformations in amphibians, (2) determine whether malformed amphibians still occur at these sites, and (3) compare the amphibian malformation patterns from these wetlands to those observed at sites discovered recently. We gathered and reviewed available information on North American wetlands that historically (1946–1988) yielded high frequencies of severe malformations in amphibians. We then obtained amphibian voucher specimens corresponding to these sites, used current terminology to redescribe observed abnormalities, and removed trematodes for comparison with known samples of *Ribeiroia*. Finally, we resurveyed these historical sites to determine the current frequency and composition of malformations. These patterns were compared with data presented in the original historical accounts and with the malformation data reported by Johnson et al. (2002) for amphibians in the western United States.

Methods

Selection of Study Sites

We obtained information on field sites associated historically with mass malformations in amphibians through published accounts, interviews with herpetologists and field biologists, and reports filed at the North American Reporting Center for Amphibian Malformations (http://www.npwrc.usgs.gov/narcam/). We contacted the original author(s) or individual(s) who made the observation for specific details regarding the site's geographic location and the disposition of voucher specimens. Emphasis was placed on sites with higher-than-baseline (>5%) frequencies of severe limb malformations and on sites for which corresponding voucher specimens could be obtained.

Voucher Specimens

Normal and abnormal amphibian specimens vouchered from identified malformation sites were generously loaned from a variety of museum and private collections (Appendix). We examined specimens with a stereoscope and described any morphological abnormalities according to current terminology (Johnson et al. 2001*b*). We dissected a subset of specimens and removed larval trematodes (metacercariae). We dissected only a limited number of voucher specimens to minimize damage to an irreplaceable resource. Our focus was on *Ribeiroia*, so we exclusively isolated metacercariae from the inguinal region and the tail resorption site because these are the predominant areas of encystment (Johnson et al. 2002).

We used serial sectioning to examine parasites from preserved amphibians because encysted trematode metacercariae can be difficult to identify, particularly after several decades of preservation. Recovered cysts were post-fixed in Bouin's fluid, dehydrated by serial passage through an ethanol series, cleared in cedarwood oil, embedded in paraffin, and sectioned at 6 μm. We stained sections in Meyer's hematoxylin and counterstained with eosin dissolved in xylene (McLean 1934, as cited in Lee 1937). We measured cyst length and width, oral sucker length and width, and acetabulum width at a magnification of 400× with an ocular micrometer. The deteriorated condition of many of the metacercariae precluded our inclusion of other features in the analysis. Due to the inconsistent orientation of metacercariae, measurements of cyst and sucker lengths showed substantial variation and were excluded from the analysis.

To determine the parasite species present at each locality, we identified metacercariae by direct observation of diagnostic characteristics, such as the esophageal diverticula found in *Ribeiroia* specimens. In some cases, direct identification was precluded by the condition or, less frequently, orientation of the metacercariae. Thus, we used parametric discriminant-function analysis (DFA) to predict the group membership of all metacercariae. Discriminant analysis is a linear modeling method that uses explanatory variables to differentiate among predefined groups (Legendre & Legendre 1998). We subjected the morphometric data from all recovered metacercariae to principle components analysis (PCA) as an objective means of verifying that no additional coherent subgroups (i.e., unidentified parasite taxa) were included in our data set. We calibrated the DFA model with metacercariae classified by direct observation as *Ribeiroia* from Hidden Pond (California, U.S.A.), Hemholtz Pond (Oregon, U.S.A.) and Ney Pond (Minnesota, U.S.A.), as *Megalodiscus* sp. from Veterans' Park (Idaho, U.S.A.), as *Fibricola* sp. from Ney Pond, and as *Glypthelmins* sp. from Phillips Bayou (Mississippi, U.S.A.). Following calibration, we used the model to identify all unknown metacercariae through linear combinations of descriptive morphological characters (discriminant functions). Analyses were performed with SAS 8.2 (SAS Institute, Cary, North Carolina).

Resurveys of Historical Field Sites

Nine sites associated with malformed amphibians in historical surveys from California, Idaho, Montana, Colorado, Texas, Mississippi, and Ohio were resurveyed between June 1999 and July 2002 (Table 1). Detailed descriptions of sampling methods are provided elsewhere (Johnson et al. 2001*b*, 2002). Metamorphic amphibians were captured by hand, measured, and inspected for morphological aberrations. At two sites known to support long-toed salamanders (*Ambystoma macrodactylum* and *A. macrodactylum croceum*), we conducted additional surveys of adult salamanders as they returned to breed. We captured metamorphic bullfrogs (*Rana catesbeiana*), green frogs (*R. clamitans*), and southern leopard frogs (*R. sphenocephala*) at night with the aid of a headlamp and dipnet. Larval amphibians were collected with a dipnet at 12 evenly spaced stations around the site's perimeter (Johnson et al. 2002). We also recorded data on the presence of aquatic snails (*Planorbella* spp.), which serve as required first intermediate hosts in the life-cycle of *Ribeiroia*. All malformed amphibians and a subset of normal individuals were collected for more detailed examinations and parasite evaluation. We released any remaining amphibians at the study site. Water samples were collected and shipped overnight to the U.S. Geological Survey Organic Geochemistry Laboratory (Lawrence, Kansas) for analysis of 61 herbicides, herbicide metabolites, and organophosphate insecticides (methods described in Johnson et al. 2002).

Within 6 hours of collection, we dissected three to five amphibians of each species from a site. We focused our attention on stages of *Ribeiroia* because it is the only trematode known to cause amphibian malformations. Living metacercariae were counted, excysted mechanically, and identified. For each site, we calculated the mean infection intensity as the average number of *Ribeiroia* metacercariae per infected amphibian. Samples of each planorbid snail species were dissected and examined for trematode rediae and cercariae. We identified cercariae and metacercariae of *Ribeiroia* by the presence of esophageal diverticula, sinuous concretions, and other features described by Yamaguti (1975).

Results

Historical Malformation Patterns

Of the nine historical sites we examined, three supported accounts of malformations from multiple years or species (Table 1). Surviving original authors and observers were located and interviewed for eight of the nine sites (see acknowledgments). Four additional accounts of deformed amphibians were excluded owing to inadequate information about the site's location or disposition of voucher specimens (i.e., Storer 1925; Miller 1968; Merrell 1969; Worthington 1974).

Examination of malformed voucher specimens in combination with information from published reports and interviews identified the following trends: (1) extra limbs (polymelia) and digits (polydactyly) were the most common types of abnormalities, (2) malformations predominantly affected the hind limbs, and (3) all malformations involved larval or metamorphic amphibians (Tables 2 & 3). Redescriptions of original voucher specimens often revealed a more serious array of malformations than described historically. For example, the leopard frog described by Rosine (1955) as suffering from polydactyly and a "badly deformed right hindlimb" actually exhibited moderate cutaneous fusion and emaciation in the right hind limb, with a distinct bony triangle in the tibiofibula of the left hind limb (University of Colorado Museum 9971).

Jette Pond, Dersch Meadows, Suburban Pond, Seascape, and Veterans' Park each supported Pacific treefrog (*Hyla regilla*) populations with a high frequency (20–72%) of hind-limb deformities. Individual frogs with two or more extra hind limbs were not uncommon among vouchered specimens, and Sessions and Ruth (1990) reported a Pacific treefrog with 10 supernumerary hind limbs. A broad array of malformation types was observed, but skin webbings, extra hind limbs (Fig. 1), and femoral projections constituted the dominant majority among all populations and years (Table 2).

At Muskee Lake and Seascape, salamanders exhibited a high frequency of extra limbs and digits (Fig. 2), primarily

Table 2. Composition of morphological abnormalities in Pacific treefrogs (*Hyla regilla*) from historical sites supporting *Ribeiroia* and mass malformations, as derived from original reports, resurvey data, and archived museum specimens.

Abnormality type	Jette Pond[a] 1958–1961, 1964 n (%)	Jette Pond 1999–2001 n (%)	Suburban Pond[b] 1981 n (%)	Seascape[c] 1986–1987 n (%)	Seascape 1999 n (%)	Veterans' Park[d] 1988 n (%)	Dersch Meadows 1999 n (%)	Western U.S.A.[e] 1996–1999 n (%)
Cephalic and axial	0	0	0	0	4 (33.4)	0	5 (7.9)	86 (2.8)
Forelimb	0	2 (0.6)	0	1 (0.3)	0	0	0	44 (1.4)
Hindlimb								
ectrodactyly	0	4 (1.2)	1 (6.7)	—[f]	1 (8.3)	2 (6.3)	6 (9.5)	109 (3.5)
polydactyly	0	5 (1.6)	3 (20)	—[f]	0	1 (3.1)	4 (6.3)	82 (2.6)
ectro- and hemimelia	0	1 (0.3)	0	43 (14.2)	1 (8.3)	1 (3.1)	5 (7.9)	2258 (7.2)
polymelia and polypody	20 (87.0)	176 (54.8)	9 (60)	234 (77.2)	2 (16.7)	14 (43.8)	18 (28.6)	1497 (47.9)
femoral projection	1 (4.3)	50 (15.6)	2 (13.3)	—[f]	3 (25)	6 (18.8)	10 (15.9)	416 (13.3)
cutaneous fusion	2 (8.7)	70 (21.8)	0	25 (8.3)	0	6 (18.8)	3 (4.8)	366 (11.7)
taumelia	0	4 (1.2)	0	—[f]	0	0	6 (9.5)	108 (3.5)
micromelia	0	4 (1.2)	0	—[f]	1 (8.3)	0	1 (1.6)	34 (1.1)
other[g]	0	5 (1.6)	0	0	0	2 (6.3)	5 (7.9)	163 (5.2)
No. abnormal	14	147	13	202	10	21	51	1282
No. abnormalities	23	321	15	303	12	32	63	3130
No. inspected	86	252	54	280	77	23	720	15455
Severity[h]	1.64	2.18	1.15	1.5	1.2	1.52	1.24	1.50

[a] *Hebard & Brunson 1963; Smithsonian Herpetological Collection 334530–42, 334545.*
[b] *Table 1 in Reynolds and Stephens (1984) and one preserved specimen. "Midcaudal bleb" presented here as "femoral projection," "complete polymelia" as "polymelia," and "multiple polyemlia" as two "polymelia" (T. Stephens 1999, personal communication).*
[c] *Sessions & Ruth 1990.*
[d] *Preserved specimens courtesy of T. Stephens.*
[e] *Johnson et al. 2002 and unpublished data.*
[f] *These categories could not be assessed based on the format of the data.*
[g] *Includes brachydactyly, clinodactyly, and limb hyperextension.*
[h] *Mean number of abnormalities per abnormal frog.*

in the hind limbs, but >300 salamanders from Seascape exhibited polydactyly in a forelimb (Table 3). Juvenile bullfrogs with between one and four extra hind limbs were reported from both Morgan Pond and Phillips Bayou (Duncan 1958; Anonymous 1962; Volpe 1977). Each of the specimens we examined from these sites exhibited two well-developed supernumerary hind limbs articulating with the pelvic girdle. Ripley Pond also supported deformed bullfrogs, but the malformations were strikingly diverse and much more severe. The total number of extra hind limbs among the nine specimens examined was 40; one frog possessed 10 hind limbs, whereas five other frogs had ≥7. In many cases, the limbs were poorly developed and completely independent of the appendicular skeleton, originating ventrally from abdominal tissue between the pelvic and pectoral girdles (gastromelia). Hauver (1958) reported similarly severe malformations in bullfrogs from the same site, ranging from complete suppression of a hind limb to five or more extra limbs.

Identification of Parasites from Voucher Specimens

We examined and dissected voucher specimens from each site except Morgan Pond, for which the malformed frogs collected in 1961 were preserved as skeletons only, and Seascape, from which patterns were inferred from data presented by Sessions and Ruth (1990) and personal communications. Trematode metacercariae were found in preserved amphibians from each site except Ripley Pond. We directly identified *Ribeiroia* metacercariae from five of the six remaining sites, including Muskee Lake in 1950, Jette Pond in 1959, Dersch Meadows in 1975, Suburban Pond in 1981, and Veterans' Park in 1988. Metacercariae of *Glypthelmins* sp. were identified in samples from Dersch Meadows and Jette Pond, whereas amphistome metacercariae (presumably *Megalodiscus* sp.) were recovered from both Idaho localities.

Classification of metacercariae by DFA confirmed the presence of *Ribeiroia* at the five sites from which we identified the parasite directly (Table 4). Three of these sites supported *Ribeiroia* in voucher specimens from multiple years, further verifying the results (Table 4). We did not detect *Ribeiroia* among samples from Phillips Bayou, either by observation or DFA. None of the other parasite groups was observed at more than three sites (Table 4). Amphistome and *Ribeiroia* metacercariae were the most difficult to distinguish by DFA classification. The generalized squared distances between trematode metacercariae were as follows: *Ribeiroia* and *Megalodiscus*, 2.403; *Ribeiroia* and *Fibricola*, 29.275; *Ribeiroia* and *Glypthelmins*, 16.707; *Megalodiscus* and *Fibricola*, 27.446; *Megalodiscus* and *Glypthelmins*, 28.033; and *Fibricola* and *Glypthelmins*, 34.898. Two of 12 known amphistome metacercariae were classified incorrectly as *Ribeiroia*, and 12 of 57 known *Ribeiroia* were classified incorrectly as amphistomes by DFA. The remaining 55 samples were classified correctly. However, amphistome

Table 3. Composition of morphological abnormalities in amphibians from historical sites with mass malformations, as derived from original reports, resurvey data, and archived museum specimens.

Abnormality type	*Muskee Lake*[a] *1946, 1950–1951* Ambystoma tigrinum *n (%)*	*Ripley Pond*[b] *1954–1955* Rana catesbeiana *n (%)*	*Seascape*[c] *1986–1987* A. macrodactylum croceum *n (%)*	*Seascape*[d] *1998–2000* A. macrodactylum croceum *n (%)*	*Jette Pond 1998–2002* A. macrodactylum *n (%)*
Cephalic and axial	0	1 (1.5)	0	0	1 (1.3)
Forelimb					
ectrodactyly	1 (2.5)	0	36 (2.1)	6 (11.5)	4 (5.0)
syndactyly	0	0	2 (0.1)	5 (9.6)	3 (3.8)
polydactyly	1 (2.5)	0	339 (19.8)	12 (23.1)	4 (5.0)
ectro- and hemimelia	0	0	3 (0.2)	5 (9.6)	1 (1.3)
polymelia and polypody	0	0	1 (0.1)	0	7 (8.8)
femoral projection	0	0	0	0	2 (2.5)
other[e]	0	0	4 (0.2)	2 (3.8)	2 (2.5)
Hindlimb					
ectrodactyly	2 (5.0)	2 (2.9)	72 (4.2)	2 (3.8)	4 (5.0)
syndactyly	0	0	11 (0.6)	7 (13.5)	0
polydactyly	30 (75.0)	2 (2.9)	508 (29.7)	0	13 (16.3)
ectro- and hemimelia	0	2 (2.9)	311 (18.2)	8 (15.4)	0
polymelia and polypody	6 (15.0)	58 (85.3)	399 (23.4)	0	9 (11.3)
femoral projection	0	1 (1.5)	0	0	8 (10.0)
cutaneous fusion	0	0	0	0	18 (22.5)
other[e]	0	2 (3.0)	22 (1.3)	5 (9.6)	2 (2.5)
No. abnormal	24	16	1686	52	61
No. abnormalities	40	68	1708	522	80
No. inspected	n/a	250+	5926	5806	524
Severity[f]	1.67	4.25	1.01	1.0	1.31

[a] *Bishop (1947), Bishop and Hamilton (1947), UCM 9863-7.*
[b] *HZM AR648-53, AR163.*
[c] *Sessions and Ruth (1990).*
[d] *Laabs (2000, 2001).*
[e] *Includes brachydactyly, clinodactyly, and limb hyperextension.*
[f] *Mean number of abnormalities per abnormal frog.*

metacercariae are easily distinguishable by direct observation and were observed only among the Idaho samples, limiting the potential for misclassification. Thus, the classification of 10 metacercariae by DFA from Muskee Lake as amphistomes is probably erroneous (Table 4).

Resurveys of Historical Field Sites

Of the nine resurveyed historical sites, one site had been destroyed (Suburban Pond), whereas two others no longer supported breeding amphibian populations (Muskee Lake [now "Mud Lake"] and Veterans' Park). Three of the remaining sites—Jette Pond, Seascape, and Dersch Meadows—continued to support amphibians with severe limb malformations at frequencies of 7–50% in one or more species (Table 5). Amphibians from each of these sites were infected heavily with *Ribeiroia* metacercariae (Table 5), which were usually aggregated around the base of the hind limbs in anurans and around both limb pairs in urodeles. We did not detect any of 61 pesticides and pesticide metabolites for which we analyzed, despite detection limits of 0.05 μg/L. Malformations observed in *H. regilla* were dominated by extra limbs (Fig. 2), femoral projections, and cutaneous fusions and were similar to both the malformations recorded in this species historically and to those associated recently with *Ribeiroia* infection from sites across the western United States (Table 2). We observed *Planorbella* spp., the snail intermediate host for *Ribeiroia*, at all three sites (*P. tenuis*: Seascape; *P. subcrenatum*: Jette Pond, Dersch Meadows). Snails infected with rediae and cercariae of *Ribeiroia* were recorded at Jette Pond and Dersch Meadows. Although no planorbid snails were observed at Muskee Lake in 1999, museum collections indicate that *Planorbella subcrenatum* was present historically (University of Colorado Museum 34528). Their disappearance could be a consequence of introduced crayfish (*Pacifastacus leniusculus*), which were observed during our resurvey and are known to reduce snail abundance (Nyström et al. 2001). One planorbid snail (*P. subcrenatum*) was also observed at Veterans' Park.

The frequency of malformations in newly metamorphosed Pacific treefrogs from Jette Pond was significantly greater in 1999–2002 than during the late 1950s and early 1960s (*G* test, $p < 0.001$; Table 5). Moreover, between 1998 and 2002 severe malformations were also observed in long-toed salamanders and western toads (*Bufo boreas*), species that were apparently unaffected

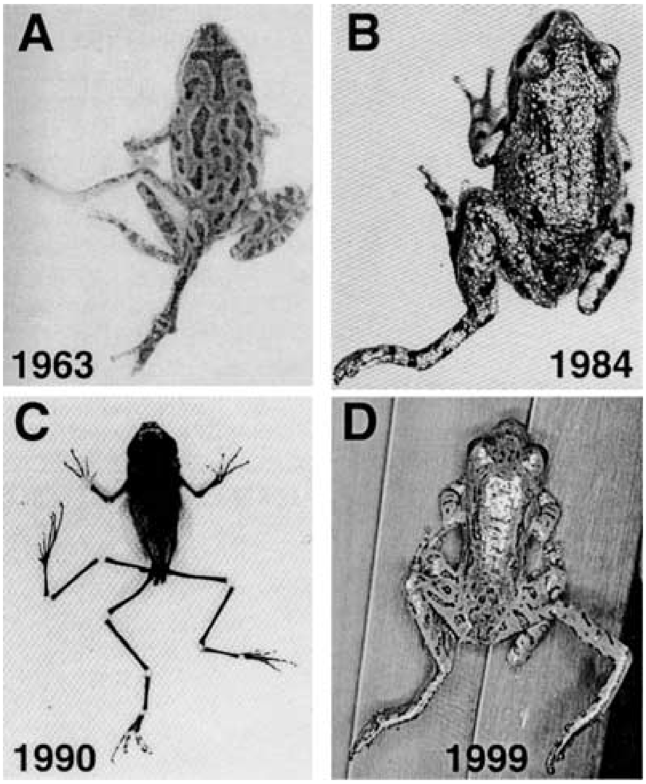

*Figure 1. Malformations in Pacific treefrogs (*Hyla regilla*) from historical and recent surveys: (a) Jette Pond, Montana (Hebard & Brunson 1963); (b) Suburban Pond, Idaho (Reynolds & Stephens 1984); (c) cleared and stained specimen from Seascape, California (Sessions & Ruth 1990); (d) specimen collected in California in 1999.*

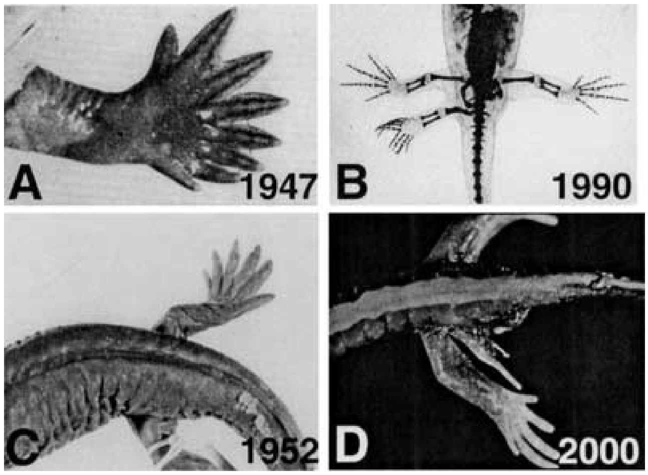

*Figure 2. Malformations in salamanders from historical and recent surveys: (a) and (c) tiger salamander (*A. tigrinum*) from Muskee Lake with polydactyly; (b) long-toed salamander (*A. macrodactylum croceum*) from Seascape with an extra hind limb (Sessions & Ruth 1990); (d) long-toed salamander with an extra hind limb outgrowth collected from Jette Pond in 2000.*

in 1958 (Hebard & Brunson 1963). Surveys in April of 1998, for instance, showed that 34 of 271 (13%) returning adult long-toed salamanders had malformations, whereas 15 of 43 (35%) of larvae were malformed in 2001. Malformations included extra digits and limbs in the fore- and hind limbs, similar to deformities reported historically from Seascape and Muskee Lake (Fig. 2; Table 3). Among metamorphosing western toads in 2002, we observed polymelia, cutaneous fusions, and taumelia, the three most common deformities induced in laboratory infections of western toad larvae with *Ribeiroia* (Johnson et al. 2001*a*). At Seascape, however, the frequencies of limb malformations in treefrogs and in Santa Cruz long-toed salamanders were each significantly lower in 1999–2001 than in 1986–1987 (Table 5; *G* test, $p < 0.0001$). Historical data for Dersch Meadows were insufficient to evaluate a possible change in the frequency of malformations.

We observed no serious malformations at either Ripley or Morgan ponds, and too few frogs were available at Phillips Bayou to evaluate the frequency of malformations accurately. At Ripley Pond, 1 of 25 metamorphosing bullfrogs exhibited a split digit in the left foot, whereas 1 of 61 leopard frogs from Morgan Pond suffered an injured forelimb. Metacercariae of *Ribeiroia* were not recovered among the frogs dissected from these sites, but several other trematode species (e.g., *Manodistomum* sp. and *Fibricola* sp.) were observed. We did not observe planorbid snails at Ripley Pond or Phillips Bayou, but several empty shells (*Planorbella* sp.) were found at Morgan Pond. In the absence of more severe malformations, we did not analyze water samples from these sites for pesticides.

Discussion

Although mass malformations are not new to North American amphibians (Bishop 1947; Hauver 1958; Hebard & Brunson 1963; Volpe 1977; Reynolds & Stephens 1984), they were infrequently reported and rarely investigated prior to 1990. Many agents are known to cause amphibian abnormalities in laboratory experiments (reviewed in Ouellet 2000), but attempts to link these factors to malformations in natural populations are often unsuccessful. Our results indicate that many historical cases of mass malformations in amphibians are associated with trematode (*Ribeiroia*) infection. We isolated metacercariae of *Ribeiroia* from six of the eight sites with vouchered specimens, dating back as far as 1946 and affecting four amphibian species from four states. Based on the results of Johnson et al. (2002), which demonstrated a significant

Table 4. Classification of trematode metacercariae from vouchered amphibian specimens by discriminant function analysis.

Locality	*Number of metacercariae classified as:* Ribeiroia	Megalodiscus	Fibricola	Glypthelmins	*Total*
Dersch Meadows 1952	2	0	0	0	2
Dersch Meadows 1974–1975	11	0	0	0	11
Muskee Lake 1946–1947	11	2	0	0	13
Muskee Lake 1950	10	10	0	0	20
Muskee Lake 1952	15	0	0	0	15
Phillips Bayou 1958	0	0	0	7	7
Jette Pond 1959	14	2	0	4	20
Jette Pond 1964	2	0	0	0	2
Suburban Pond 1981	7	0	0	0	7
Veterans' Park 1988	8	12	0	2	22

functional relationship between *Ribeiroia* infection and amphibian malformations, we consider it unlikely that the presence of *Ribeiroia* in these historical specimens is coincidental. The malformations recorded at these sites, both recently and historically, were consistent with those produced by *Ribeiroia* in the laboratory (Johnson et al. 1999, 2001*a*; Stopper et al. 2002) and field studies (Johnson et al. 2001*b*, 2002; Kiesecker 2002), including extra limbs (Figs. 1 & 2), missing limbs, cutaneous fusion, and bony triangles. Moreover, all of the sites associated historically with *Ribeiroia* that continued to support amphibian populations also continued to support *Ribeiroia* and above-baseline (>5%) frequencies of malformations, further implicating *Ribeiroia* infection in the malformations observed historically. Indeed, at Jette Pond, malformed treefrogs were reported in 1958–1961, 1964, 1975, and now in 1999–2002. The long-term consequences of infection and malformations are unclear, but populations of both *Rana pipiens* and *R. luteiventris* have disappeared from Jette Pond since the 1970s.

Neither *Ribeiroia* metacercariae nor any serious malformations were observed during resurveys of Ripley Pond, Morgan Pond, and Phillips Bayou. Each of these cases involved bullfrogs with extra hind limbs observed in the 1950s or early 1960s. In contrast to the sites associated with *Ribeiroia*, malformations in these wetlands were observed for 1–2 years and then disappeared completely (Hauver 1958; Volpe 1977, personal communication;

Table 5. Comparison of recent (1999–2002) and historical (1946–1988) survey data on amphibian malformation patterns and *Ribeiroia* infection at North American wetlands.[a]

Site	*Year*	*Species*	*Resurvey date*	*Frequency of malformations* historical % (total number sampled)	resurvey	Ribeiroia *infection intensity* historical no. metacercariae ± 1 SE	resurvey
Muskee Lake	1946–1947, 1950–1951	*A. tigrinum*	16 August 1999	90 (19)	extirpated	4.0 ± 3.1	—
Muskee Lake	1950–1951	*R. pipiens*	16 August 1999	(3)	extirpated	33.8 ± 18.1	—
Ripley Pond	1954–1955	*R. catesbeiana*	23 August 2001	~10 (250+)	3.6 (28)	0	0
Phillips Bayou	1958	*R. catesbeiana*	12 August 2001	~50 (200+)	n/a	0	0
Jette Pond	1958–1961, 1964	*H. regilla*	August 1999–2002	20 (86)	46.3 (337)[b]	38.5 ± 11.5	52.7 ± 12.1
Jette Pond	1958–1961	*A. macrodactylum*	April 1998–2002	0	10.9 (524)	n/a	28.4 ± 8.8
Jette Pond	1958–1961	*B. boreas*	29 July 2002	0	6.1 (49)	n/a	n/a
Morgan Pond	1961	*R. catesbeiana*	29 July 2001	~5 (200+)	extirpated	n/a	—
		R. sphenocephala	29 July 2001	—	1.7 (60)	—	0
Dersch Meadows	1974	*H. regilla*	3 September 1999	"many"	7.1 (720)	n/a	21.4 ± 5.3
Dersch Meadows	1952, 1974–1975	*R. cascadae*	3 September 1999	n/a	extirpated	5.0 ± 1.9	—
Suburban Pond	1981	*H. regilla*	13 August 1999	22 (54)	destroyed	23.0	—
Seascape	1986–1987	*H. regilla*	14 June 1999	72 (280)	13 (77)[b]	present	4.0 ± 1.5
Seascape	1986–1987	*A. macrodactylum croceum*	April 1998–2000	28 (5926)	0.9 (5806)[b]	—[c]	—[c]
Veterans' Park	1988	*H. regilla*	13 August 1999	"many"	extirpated	25.5 ± 4.2	—

[a] *Abbreviation: n/a, insufficient data.*
[b] *Represents significant change from historical value* (R × C *test*, $p < 0.001$).
[c] *Endangered status precluded collection of this species.*

J. Morgan, personal communication). Although each of the wetlands was an artificial impoundment, they varied widely in age, size, and surrounding land use. Ripley and Morgan ponds provided water for livestock, whereas Phillips Bayou bordered cotton fields and later a plant nursery. Historically, investigators of the phenomena speculated that radioactivity, pesticides, or genetic mutations might be responsible but offered little supporting evidence (Table 1). The absence of malformations during our resurveys reduces the likelihood of follow-up investigations providing any further insights, and the causative agent(s) at these sites may never be known.

Comparison of Recent and Historical Malformations

Unfortunately, three of the nine historical sites included in our study no longer supported amphibians. One site had been filled and two had experienced introductions of exotic fishes (Veterans' Park) or crayfish (Muskee Lake), possibly contributing to the loss of amphibian inhabitants. At the three sites that continued to support malformed amphibians, no clear pattern was observed. The frequency of malformations at Jette Pond in 1999–2002 was significantly greater than in 1958–1961. Amphibians from Seascape exhibited a significantly lower malformation frequency in 1999–2001 than in 1986–1987, and the historical data from Dersch Meadows were insufficient to evaluate changes. Although the number of sites and sampling years are too few to make conclusions, the site that declined in frequency (Seascape) is a natural preserve managed to protect Santa Cruz long-toed salamanders, whereas Jette Pond, which showed an increase in malformations, has served as a cattle watering hole for more than 50 years and has been further degraded by logging (Miller 1975). Consequently, the pond is highly eutrophic and supports a dense population of *Planorbella subcrenatum*, which may have contributed to the growing numbers of malformations by elevating the number of first intermediate hosts available to *Ribeiroia* (Johnson & Lunde 2004).

Based on our review of mass-malformation accounts involving North American amphibian populations and our redescription of malformed voucher specimens, many of the malformations observed recently have historical precedent. Severe malformations, such as cutaneous fusion, bony triangles, missing limbs, and extra limbs, have been observed since the 1950s, sometimes at frequencies of 50% or greater, but were often described inadequately. Indeed, the terms *cutaneous fusion* and *bony triangle* have only recently come into usage (Gardiner & Hoppe 1999; Hoppe 2000). Cutaneous fusion was among the most prevalent malformations observed recently at certain Minnesota field sites but was absent in museum vouchers from the same region (Hoppe 2000). We recorded cutaneous fusion in frogs from Muskee Lake, Jette Pond, and Veterans' Park dating back to 1950. Some historical malformations even surpass recent observations in their severity. For example, vouchered bullfrogs from Ripley Pond were so severely deformed, some with as many as eight extra limbs emerging from the abdomen (HZM MU#A649), that their description challenged even our current terminology.

An Emerging Disease

Emerging diseases are defined as diseases whose occurrence, geographic distribution, host range, or pathenogenicity has increased substantially over the past three decades (Centers for Disease Control and Prevention 1994; Friend et al. 2001). The last 30 years have witnessed an unprecedented rate of disease emergence and re-emergence among both human and wildlife populations (Morse 1995; Daszak et al. 2000). Although some of the pathogens responsible represent novel forms, most have existed for some time, and their emergence is a consequence of environmental change (Fayer 2000; Dobson & Foufopoulos 2001). For example, Lyme disease, currently the most common tick-borne zoonosis in the Unted States, was unrecognized prior to 1975. Examinations of vouchered tick specimens, however, revealed evidence of the spirochete infection as far back as the 1940s (Persing et al. 1990). Lyme disease has "emerged" in recent decades as a consequence of reforestation of the northeastern United States and expanding populations of white-tailed deer (*Odocoileius virginianus*; Barbour & Fish 1993).

In a parallel manner, we suspect that malformed amphibians resulting from trematode infection have become more prevalent in recent years. Unfortunately, a rigorous test of this hypothesis is precluded by insufficient data; we have few accurate counts of mass-malformation sites, currently or historically, and no time-series data from which to detect change. Nevertheless, the combination of several pieces of circumstantial evidence suggests that, owing to *Ribeiroia* infection, malformed amphibians have become more common.

PAUCITY OF HISTORICAL MALFORMATION ACCOUNTS

Results of our exhaustive literature search identified only seven historical (pre-1990) records of mass malformations in amphibians associated with *Ribeiroia* infection. In contrast, Johnson et al. (2002) reported 25 such sites in the western United States alone, with malformations in six species at frequencies ranging from 5% to 90%. Dozens of mass-malformation sites associated with *Ribeiroia* have also been identified recently in Minnesota, Wisconsin, Illinois, and New York (P.T.J.J. & K.B.L., unpublished data; D. Sutherland, unpublished data; V. Beasley, personal communication). Although some portion of the apparent increase in amphibian malformations is undoubtedly attributable to heightened surveillance, the majority of malformation sites, recently and historically, are discovered by children, not scientists. Moreover, as with current observations, past malformation "outbreaks" also generated extensive media coverage and concern over human health (Table 1). In comparison to these isolated incidents, however, current observations represent a malformation "epidemic," with reports of new sites and affected species each season (North American Reporting Center for Amphibian Malformations [http://www.npwrc.usgs.gov/narcam/]).

EXTENSIVE HISTORICAL SURVEYS OF AMPHIBIANS

Pacific treefrogs, the species most commonly affected with malformations in both recent and historical accounts, have been studied intensively throughout their range (e.g., Storer 1925; Brattstrom & Warren 1955; Jameson 1956, 1957; Whitney & Krebs 1975; Schaub & Larsen 1978; Nussbaum et al. 1983). David Jameson and colleagues examined tens of thousands of living and vouchered treefrogs from California, Oregon, Idaho, and Montana between 1950 and 1980 but observed few malformations and no mass-malformation sites (D. Jameson & J. Mackie, personal communication). Our own examinations of nearly 2000 vouchered Pacific treefrogs also yielded few malformations (Johnson & Lunde 2004). Moreover, one of the historical malformation sites, Lassen Volcanic National Park, has been surveyed widely for its amphibians, with no records of malformations (e.g., Grinnell et al. 1930; Stebbins 1952; Sage 1974; Fellers & Drost 1993; Jennings & Hayes 1994). Between 1999 and 2000, at least four sites with severely malformed amphibians were recorded from within the park (Johnson et al. 2002; S. Zachary, personal communication).

RIBEIROIA INFECTION AND ARTIFICIAL IMPOUNDMENTS

Forty-four of 59 wetlands associated with *Ribeiroia* infection were artificial impoundments or wetlands that had been substantially altered by human activity (Johnson et al. 2002). Lannoo et al. (2003) observed a similar preponderance of "created" wetlands among malformation hotspots in the midwestern United States. The majority of these sites did not exist 50 years ago, and we suspect that they represent an expansion in the geographic prevalence of malformed amphibians. Since the 1930s, extensive pond building by agencies such as the U.S. Soil Conservation Service has led to a 1.2-million-ha increase in artificial wetlands in the United States (Tiner 1984; Dahl & Johnson 1991; Leja 1998). Unfortunately, little is known about the ecological significance of such habitats.

An increase in the prevalence of *Ribeiroia* and sites with mass malformations in amphibians may result from extensive modification of aquatic systems in North America. Human-mediated alterations to wetlands, particularly the building of impoundments (e.g., dams, reservoirs, borrow pits, stock ponds) have frequently improved snail habitat and led to outbreaks of trematode-related diseases, such as schistosomiasis, fascioliasis, paragonimiasis, and clonorchiasis (Coates & Redding-Coates 1981; Madsen & Frandsen 1989). North American farm ponds, of which 80% are built to support cattle (Bennett 1971), may be particularly favorable to *Ribeiroia* and its *Planorbella* snail hosts as a consequence of their permanent, often highly productive conditions (Johnson & Lunde 2004). *Planorbella* snails are significantly associated with eutrophic, artificial wetlands, wherein they attain a larger maximum size and a higher population density (Chase 1998; Johnson et al. 2002). Water birds, including many *Ribeiroia* definitive hosts, also frequent these habitats, particularly as natural wetlands are continually eliminated. Additional insults, such as certain pesticides or ultraviolet radiation, may impair amphibians' immune resistance to *Ribeiroia* infection, further increasing the prevalence of malformations observed in recent years (Kiesecker 2002; Blaustein & Johnson 2003).

Conservation Implications

The connection between malformations and declines in North American amphibian populations remains unexplored (Wake 1998; Hoppe 2004). However, infection by *Ribeiroia* probably represents a significant source of mortality in some populations. Larval anurans exposed to *Ribeiroia* frequently die prior to metamorphosis, and those that do emerge are often malformed (Johnson et al. 1999, 2001*a*). Malformed amphibians rarely survive to sexual maturity, even when abundant at metamorphosis (Sessions & Ruth 1990; Johnson et al. 2001*b*). In this study, *Ribeiroia* infection and malformations were recorded in several declining amphibian species, and population extinctions were observed at four of the five sites associated with *Ribeiroia* (e.g., the disappearance of *Rana pipiens* and *A. tigrinum* from Muskee Lake since the 1970s; Table 6). The direct or indirect role of *Ribeiroia* in such declines needs to be investigated.

Although the controversy surrounding malformed amphibians has often emphasized an oversimplified dichotomy between natural factors (e.g., parasite infection, predation, inbreeding) and anthropogenic factors (e.g., pesticides, elevated UV-B radiation, hormone mimics), biotic agents such as trematode infection may interact with human activity in many forms. Our results suggest that amphibian malformations caused by *Ribeiroia* infection have occurred since at least the 1940s. However, there is qualitative evidence suggesting that the number of populations affected has increased in recent years, and we emphasize the following research priorities: (1) broad-scale surveys to determine the prevalence of sites supporting *Ribeiroia* and malformed amphibians, (2) experiments exploring the interaction between *Ribeiroia* infection and secondary factors such as eutrophication, contaminants, and UV-B radiation, and (3) long-term monitoring

Table 6. Vouchered amphibian specimens examined for malformations and trematode infection.

Site	*Species*	*Year*	*Collector*	*Accession no.*	*Museum**
Muskee Lake	*Ambystoma tigrinum*	1947–1963	R. Hamilton, R. Gregg, R. Macsalka	5815, 5819, 5822–23, 5826–28, 5832, 5834–35, 47717–19, 47721, 47723–25, 51748	UCM
Muskee Lake	*Rana pipiens*	1946–1959	H. Rodeck, R Hamilton, Beidelman, Wright	1071–76, 1119, 10336–40, 11896, 51803–06	UCM
Muskee Lake	*Rana pipiens*	1950	W. Rosine	9971	UCM
Muskee Lake	*Ambystoma tigrinum*	1950–1951	W. Rosine	9863–67	UCM
Ripley Pond	*Rana catesbeiana*	1954–1955	L. Campbell, P. Daniel	MU AR648–53, MU AR163	HZM
Jette Pond	*Hyla regilla*	1959	R. Brunson	334530–42	USNM
Jette Pond	*Hyla regilla*	1964	R. Anderson	334545	USNM
Phillips Bayou	*Rana catesbeiana*	1961	C. Henry	192733–34	FMNH
Phillips Bayou	*Rana catesbeiana*	1961	C. Henry, S. Faulkner		DSU
Morgan Pond	*Rana catesbeiana*	1961	R. Baldauf	81792–93	TCWC
Dersch Meadows	*Rana cascadae*	1952	R. Stebbins	57089–91, 57709–10	MVZ
Dersch Meadows	*Rana cascadae*	1974	R. Sage	148946–88	MVZ
Dersch Meadows	*Rana cascadae*	1975	S. Case	136138–43	MVZ
Suburban Pond	*Hyla regilla*	1981	T. Stephens		ISU
Veterans' Park	*Hyla regilla*	1988	T. Stephens		ISU

**Abbreviations: UCM, University of Colorado Museum; HZM, Hefner Zoology Museum; USNM, Smithsonian Herpetological Collection; FMNH, Field Museum of Natural History; DSU, Delta State University; TCWC, Texas Cooperative Wildlife Collection; MVZ, Museum of Vertebrate Zoology; ISU, Idaho State University.*

to evaluate the impacts of parasite-induced malformations on amphibian population viability.

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