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Multiple Causes for the Malformed Frog Phenomenon

Reference: Lannoo, J. J., Sutherland, D. R., Jones, P., Rosenberry, D., Klaver, R. W., Hoppe, D. M., Johnson, P. T. J., Lunde, K. B., Facemire, C., and Kapfer, J. M., **"Multiple Causes for the Malformed Frog Phenomenon,"** *ASTM STP 1443, Multiple Stressor Effects in Relation to Declining Amphibian Populations*, G. Linder, S. Krest, D. Sparling, and E. Little, Eds., ASTM International, West Conshohocken, PA, 2003.

Abstract: Progress has been made in understanding the malformed frog problem, yet we still cannot identify with assurance specific causes of malformations at particular locations. To address this problem we assembled a team of specialists and present here results on geographic distribution, water quality, parasite infection, and morphological patterns from Minnesota malformed frog sites and reference sites. Malformed frog hotspots (> 5% malformed animals) tend to occur in a broad line from northwest to southeast across Minnesota associated with the North Central Hardwoods and Driftless Area ecoregions, and are less associated with Lake Agassiz Plain, Northern Glaciated Plain, and Western Corn Belt Plain ecoregions. Few hotspots occur in the southwestern grassland and northeastern boreal forested portions of the state. There is a tendency for

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hotspots to occur at ecoregion junctions. No single water quality feature correlates with hotspots. Heavy *Ribeiroia* infections always indicate hotspots, but lesser *Ribeiroia* infections may or may not. Conversely, certain hotspots show no evidence of the presence of *Ribeiroia*. Among reference sites, two have no evidence of *Ribeiroia*. The most common hindlimb malformation type was ectromelia, followed by micromelia and the presence of spongiform bone. Limb hyperextension, amelia, and polymelia were the least common malformation types. Malformed frog hotspots are typically associated with altered wetlands and any solution to the malformed frog problem must include restoring these sites.

Keywords: amphibian declines, malformations, habitat alteration, parasites, chemical contamination, habitat restoration

Despite no longer making headline news, the malformed frog problem has not been solved. It is true that progress has been made: malformation types have been described and general causes of amphibian malformations have been identified. Yet we still cannot answer the question foremost in the public's mind: Can we identify with assurance specific causes of malformations at particular locations? It is generally suspected that various causes are probably working at different sites, either individually or in combination. This explanation does little to assuage the concerns of affected landowners, especially those with young children. (If this sounds like hyperbole, realize that at one point during the malformed frog investigation, the state of Minnesota issued bottled water to some of these families [Souder 2000]).

There are several reasons why we have failed to achieve firm answers to questions about amphibian malformations (Souder 2000). One of the most important is that modern scientific inquiry favors specialists, and it is the tendency of specialists to see what they know and ignore what they do not (Steinbeck and Ricketts 1941). Given this nature, and given the multiple causes known to cause amphibian malformations, it is unlikely that individual researchers or individual research laboratories will generate results that fully explain the broader phenomenon. One solution to this problem is to assemble a team of specialists in relevant disciplines, and we have done this. Our team was funded through the U.S. Geological Survey's Amphibian Research and Monitoring Initiative (ARMI) and the Minnesota Pollution Control Agency (MPCA) and was divided into field biology (Hoppe, Lannoo, Sutherland, Kapfer), hydrology (Rosenberry, Jones), parasitology (Sutherland, Kapfer, Johnson, Lunde), landscape ecology (Klaver), and morphology (Lannoo). We present here our results for a subset of malformed frog sites in Minnesota, reference sites in Minnesota and in northwest Iowa, as well as sites from across the country (data from Johnson, Lunde, Facemire) that have relevance to our regional data.

Methods

We identified 17 sites for study (Fig. 1; site data summarized in Table 1) using techniques detailed in Helgen et al. (1998) and U.S. Geological Survey (2001). The majority of our sites (11; BUR, CBA, CTG, CWB, DOR, HIB, HYD, NEY, ROI, SUN, TRD) are considered "hotspots" by the Minnesota Pollution Control Agency (≥ 5% of animals with malformations in any single sample). Four Minnesota sites (BLO, GEL, IWPA and MHL) represent reference sites located within the same region. Two other sites (OKB1, OKB2) are located 150 km SW from the nearest known hotspot and represent reference sites distant from the region where hotspots are prevalent.

We sampled these sites for malformed frogs and characterized the sites in terms of origin (natural, human created [hereafter referred to as created], and

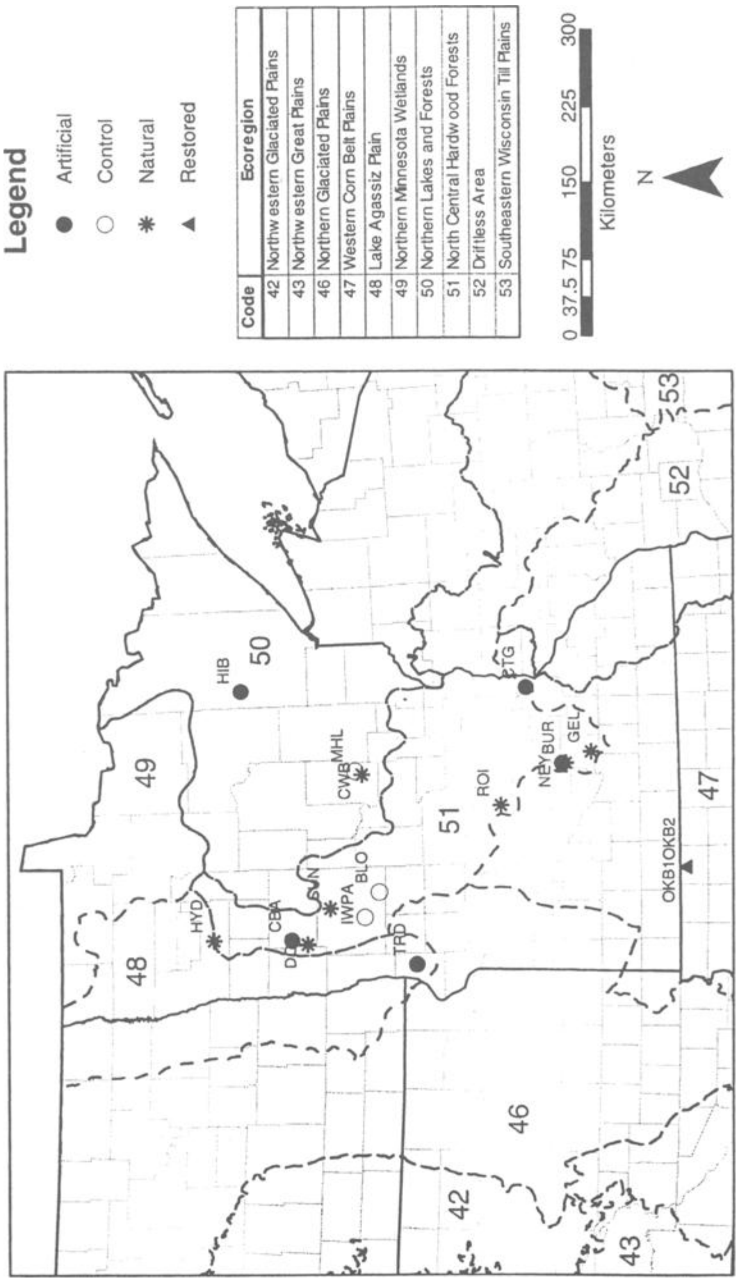

Figure 1 - *Malformed Frog Study Sites and Reference (Control) Sites Sorted by Origin (Artificial Hotspot, Control, Natural Hotspot, Restored).*

Table 1 – *Description and Classification of Each of 17 Sites Sampled for this Study.*

BLO. Semipermanent to permanent wetlands located immediately east of Block Lake, Ottertail County, Minnesota. One wetland was forested and located across a road, near lake level. A second wetland is located in an old field grassland about 200 m upland, separated from the first wetland by forested hillside. During our frog sampling visit both wetlands were nearly dry, making an assessment of macrophytes difficult. Block Lake leopard frogs have been sampled for many years by David Hoppe and Robert McKinnell because of the high frequency of burnsi morphs, which are leopard frogs exhibiting an autosomal dominant gene generally found in lower frequencies through the eastern portion of the Upper Great Plains (Merrell, 1965). Forty-four leopard frog and 11 wood frog adults were sampled. Classification: Natural, Reference site.

BUR. Small, permanent wetland in Le Sueur County, Minnesota originally chosen as a reference site for nearby NEY hotspot (see below), but malformations have appeared here. The wetland is located about 15 m from an agricultural field, which is separated from the wetland by a gravel road. The wetland is surrounded by willows (*Salix* sp.); emergent vegetation includes rushes (*Scirpus* sp.) and sedges (*Carex* sp.). The center of the wetland was mostly open water with some duckweed (*Lemna minor*). Macrophytes were sparse. Brook sticklebacks (*Culaea inconstans*) were present, as were a variety of aquatic invertebrates, including a planorbid snail (*Helosoma trivolvis*). Eighty-six leopard frog adults were sampled. Classification: Created, Hotspot.

CBA. A constructed, permanent wetland in Ottertail County, Minnesota. Wetland was made by diking a hillside punctuated with springs and fens. Water was cold (8 °C below air temperature at 1000 h), consistent with being spring fed. Dead trees were scattered along the periphery. In the water there were thick macrophytes, mostly coontail (*Ceratophyllum demersum*). Sticklebacks and mudminnows (*Umbra lima*) were present. A large number of invertebrates were also present including planorbid snails. One-hundred twenty-nine leopard frog adults and seven leopard frog tadpoles were sampled. Classification: Created, Hotspot.

Table 1 (continued)

CTG. Permanent wetland constructed to control stormwater runoff in the city of Cottage Grove, a southeastern suburb of St. Paul located in Washington County, Minnesota. The site is located in Pinetop Park, surrounded by housing developments. The wetland shoreline is partially earthen, partially paved with asphalt. Garbage, including broken lawn chairs, a television, beverage cans and (mostly broken) bottles, litters the shoreline and the wetland bottom. Rainwater enters this basin from the west. This area has fluctuating water levels and contains stands of cattails. The water is colored pea soup green with phytoplankton. Planorbid snails and clam shrimp (concostracans) were present. Twenty-six American toads (*Bufo americanus*) were sampled. Classification: Created, Hotspot.

CWB. Natural lake located near Mille Lacs Lake, in Crow Wing County, Minnesota. Wetland is surrounded by mostly deciduous forest, which in turn is interspersed with agricultural land and roadways. One lakefront house is present. Cattle from an adjacent dairy farm use a portion of the wetland and have greatly eroded the shoreline bank adjoining our collection site. Cattails (*Typha* sp.) ring the shoreline, with stands of water lilies (*Nuphar* sp.) and arrowheads (*Sagittaria* sp.) beyond the cattails, and abundant three-way sedge (*Dulichium arundinaceum*) in the areas disturbed by fallen trees and a dock. Sunfish (*Lepomis* sp.), sticklebacks, and minnows (*Pimephales* sp.) are present, as are a variety of invertebrates, including planorbid snails. The wetland is the only site visited where carcasses of frogs and fishes were observed. Eighty-eight mink frogs and one green frog (*Rana clamitans*) were sampled. Classification: Natural, Hotspot.

DOR. Large, natural, permanent wetland located in Becker County, Minnesota. Water level is generally controlled by beaver dams, but a local lake association controls against extreme fluctuations. Cattails (*Typha* sp.) ring the wetland and also occur in floating mats in deeper water. Dead trees are scattered throughout the wetland, indicating historic times when the water levels were lower. Fifty-three leopard frog adults were sampled. Classification: Natural, Hotspot.

GEL. A large permanent wetland connected to Lake Jefferson in Le Sueur County, Minnesota. Bordered by both public and private land, the wetland smelled of cattle manure. The public land is mowed to keep noxious plants especially Canada thistle

Table 1 (continued)

(*Cirsium arvense*) in check. Cattails ring the shoreline. In the water, dense and extensive mats of duckweed were present. Macrophytes include coontail and at least 2 species of pondweed (*Potamogeton* sp.), including sago pondweed (*P. pectinatus*). Bowfin (*Amia calva*) and green sunfish (*Lepomis cyanellus*) were seined from the water. Fifty leopard frogs and 19 American toads were sampled. Classification: Natural, Reference site.

HIB. A small, constructed permanent wetland in St. Louis County, Minnesota, built in the shape of a ring (to facilitate ice skating) in 1996. Lawn grades to the pond edge, some rushes, sedges, and cattails are present. In the water, sago pondweed predominates. Snails were observed but were not sampled. One American toad, seven wood frogs, and 11 leopard frogs were sampled. Classification: Created, Hotspot.

HYD. Permanent wetland in Polk County, Minnesota, ringed with cattails, willows, swamp milkweed (*Asclepias incarnata*) and Canada thistle. Agricultural fields are more distant. Site is unusual in that the bottom is covered with 50–70 cm of loose muck, likely representing erosion from adjacent fields. Frogs were not found in association with the wetland but instead were found feeding in a mowed field across a gravel driveway. Sixty-five leopard frogs were sampled here. Classification: Natural, Hotspot.

IWPA. Small, semi-permanent wetland on state property in Ottertail County, Minnesota, ringed and dotted with cattails and sedges, duckweed and star duckweed (*Lemna trisulca*) in open water. The basin is surrounded by restored prairie with little bluestem (*Andropogon scoparius*) predominant; woods were more distant. Eight wood frogs and 11 leopard frogs were sampled. Classification: Natural, Reference site.

MHL. Lake located in Crow Wing County, Minnesota that served as a reference site for CWB (see above). Includes a sphagnum bog on the southeast side. The lakeshore is wooded and partially developed with cabins. Sunfish (*Lepomis* sp.) were observed in the water. Four mink frogs and nine green frogs were sampled. Classification: Natural, Reference site.

Table 1 (continued)

NEY. Large, constructed wetland located in Le Sueur County, Minnesota and surrounded by old fields and agricultural fields now associated with a nature center. Sago pondweed predominates macrophytes and forms thick beds that restrict water currents and tend to produce pockets of warm water. Snails predominate. One-hundred fifteen leopard frogs were sampled. Classification: Created, Hotspot.

OKB1. Large, semipermanent wetland restored about 5 years ago under the U.S. Fish and Wildlife Service's Waterfowl Production Area program located 0.8 km west of Welch Lake in Dickinson County, Iowa. Ringed by cattails with a variety of macrophytes and invertebrates. Planorbid snails were present. Nine American toads and 20 chorus frogs (*Pseudacris triseriata*) were sampled. Classification: Restored, Reference site.

OKB2. Small, semipermanent wetland restored about 5 years ago under the U.S. Fish and Wildlife Service's Waterfowl Production Area program located 0.8 km west and 0.8 km south of Welch Lake in Dickinson County, Iowa. This site is ringed by cattails with a large cattail stand along the west side. Several species of macrophytes and invertebrates, including planorbid snails, were present. Eleven leopard frogs were sampled. Classification: Restored, Reference site.

ROI. Shallow, semipermanent natural wetland in Meeker County, Minnesota. The basin is surrounded by forest on the north and west sides and by mobile homes and lawns on the south and the east sides. More trash was found here than at any site except CTG. Duckweed covered over 80% of the water's surface. Emergent cattails and grasses were present, indicating a history of drying. A large number of invertebrate species were observed. One American toad, 11 leopard frogs, and four wood frogs were sampled. We also captured a gray treefrog (*Hyla versicolor*) tadpole and one salamander in the *Ambystoma laterale* complex. Classification: Natural, Hotspot.

SUN. Deep fringing wetlands associated with Paul Lake in Ottertail County, Minnesota. The wetlands are situated partially in woods, partially in the open and are gradually being filled and used for expensive lakeshore housing. The remaining habitat appeared healthy with a large number of invertebrates. Eleven leopard frog and nine

Table 1 (continued)

wood frog adults were sampled. Classification: Natural, Hotspot.

TRD. Small but deep constructed wetland on public school grounds located in Traverse County, Minnesota. The basin is composed of hardpan clay with steep sides, ringed by cattails. Well water is pumped in periodically to prevent pond drying. In August, during our sampling visit, few macrophytes were established in the open water, although by September coontail was dense (Hoppe, personal observations). Few invertebrates were observed and in three years of sampling, no snails of any species have been collected (Hoppe, personal observations). Various species of fish, including bullheads (*Amieurus* sp.) and fathead minnows (*Pimephales promelas*) have been introduced, although no fish were observed or captured in 2001 (Hoppe, personal observations). Two American toads and 14 leopard frogs were sampled. Classification: Created, Hotspot.

restored), and malformed frog history (hotspot or reference site; below, see also Table 1). A subsample of frogs from each site was collected (Minnesota Department of Natural Resources Special Use Permit No. 10510), dissected for parasites (Johnson et al. 2002), then radiographed (Table 2; Lannoo 2000). Our team of hydrologists also sampled these sites using a battery of water quality tests, including major ion composition (Brinton et al. 1996, Mitko and Bebek 2000), nutrients (Antweiler et al. 1993), and dissolved organic carbon (Wershaw et al. 1983, http://water.usgs.gov/owq; and below). These various datasets were then summarized and analyzed with reference to each other.

Results

Sample Sites

From the 17 sites visited (Table 1) we sampled a total of 837 amphibians, 274 of which were subsampled for parasite and radiographic analyses (Table 2).

Geographic Distribution —During the course of our sampling, it became apparent that hotspots are brought to the attention of authorities when there is a congruence of amphibian malformations and humans interested in the outdoors. This occurs with school group and scouting group trips, and in areas where people enjoy wildlife (see also Souder 2000). We found one previously unreported hotspot (9.1% malformation frequency) simply by being curious about a roadside wetland. Therefore, we must view this map as the product of a non-systematic sampling effort. If we believe the map to be generally representative (and there is independent evidence of this, see USGS 2001 and NARCAM 2001 for similar patterns) malformed frog hotspots tend to occur in a broad line from northwest to southeast across Minnesota—that is, few reported hotspots occur in the southwestern grassland and northeastern boreal forested portions of the state (Fig. 2). One way to characterize this pattern is through the use of Level 3 Ecoregions (originally defined by Omernik 1987; but since revised [see U.S. Environmental Protection Agency 1999]). In this analysis, hotspots are more associated with the North Central Hardwoods and Driftless Area ecoregions, less associated with Lake Agassiz Plain, Northern Glaciated Plain, and Western Corn Belt Plain ecoregions (Fig. 2). There may be some tendency for hotspots to occur at the junctions of recognized ecoregions (Fig. 2).

Water Quality Data —Few distinguishable patterns emerged with respect to a ranking analysis of the twenty-two water quality features that were considered (Table 3). No single water quality feature was related to hotspots. A few conclusions can be drawn, however. For example, water conductivity was highest in two northwest constructed wetlands (CBA, TRD) and lowest in two northeast natural wetlands (CWB, MHL). Similarly, alkalinity was highest in one northwest constructed wetland (CBA) and one northwest natural wetland (IWPA), lowest in two northeast natural wetlands (CWB, MHL). CWB and MHL also had the lowest calcium, magnesium, and sodium values.

Hotspots versus reference sites, and natural versus artificial sites could not be distinguished based on measures of pH, nitrogen, phosphorus, dissolved organic carbon, hardness, potassium, chloride, sulfate, fluoride, silica, iron, manganese, or dissolved solids.

As a second attempt to determine patterns in the water quality data, we identified the two highest and two lowest values for each feature measured and asked which wetlands were notable for these outlying values. IWPA (natural, reference site) had the highest number of outliers (10 out of 22 possible) followed by CBA (9; created, hotspot)

Table 2 – *Numbers of Anurans Examined, Sorted by Species, from Each Wetland Site. Numbers in Parentheses are the Animals Kept, Dissected for Parasites, and Radiographed. See Table 1 for Full Site Names and Descriptions.*

Site	*Bufo americanus*	*Pseudacris triseriata*	*Rana clamitans*	*Rana pipiens*	*Rana septentrionalis*	*Rana sylvatica*	Total
BLO				44 (10)		11 (10)	55 (20)
BUR				86 (12)			86 (12)
CBA				136 (13)			136 (13)
CTG	26 (26)						26 (26)
CWB			1 (1)		88 (9)		89 (10)
DOR				53 (9)			53 (9)
GEL	19 (10)			50 (10)			69 (20)
HIB	1 (1)			11 (11)		7 (7)	19 (19)
HYD				65 (10)			65 (10)
IWPA				11 (11)		8 (8)	19 (19)
MHL			9 (9)		4 (4)		13 (13)
NEY				115 (11)			115 (11)
OKB1	9 (9)	20 (20)					29 (29)
OKB2				11 (11)			11 (11)
ROI	1 (1)			11 (11)		4 (4)	16 (16)
SUN				11 (11)		9 (9)	20 (20)
TRD	2 (2)			14 (14)			16 (16)
Total	67 (49)	20 (20)	10 (10)	618 (144)	92 (13)	30 (38)	837 (274)

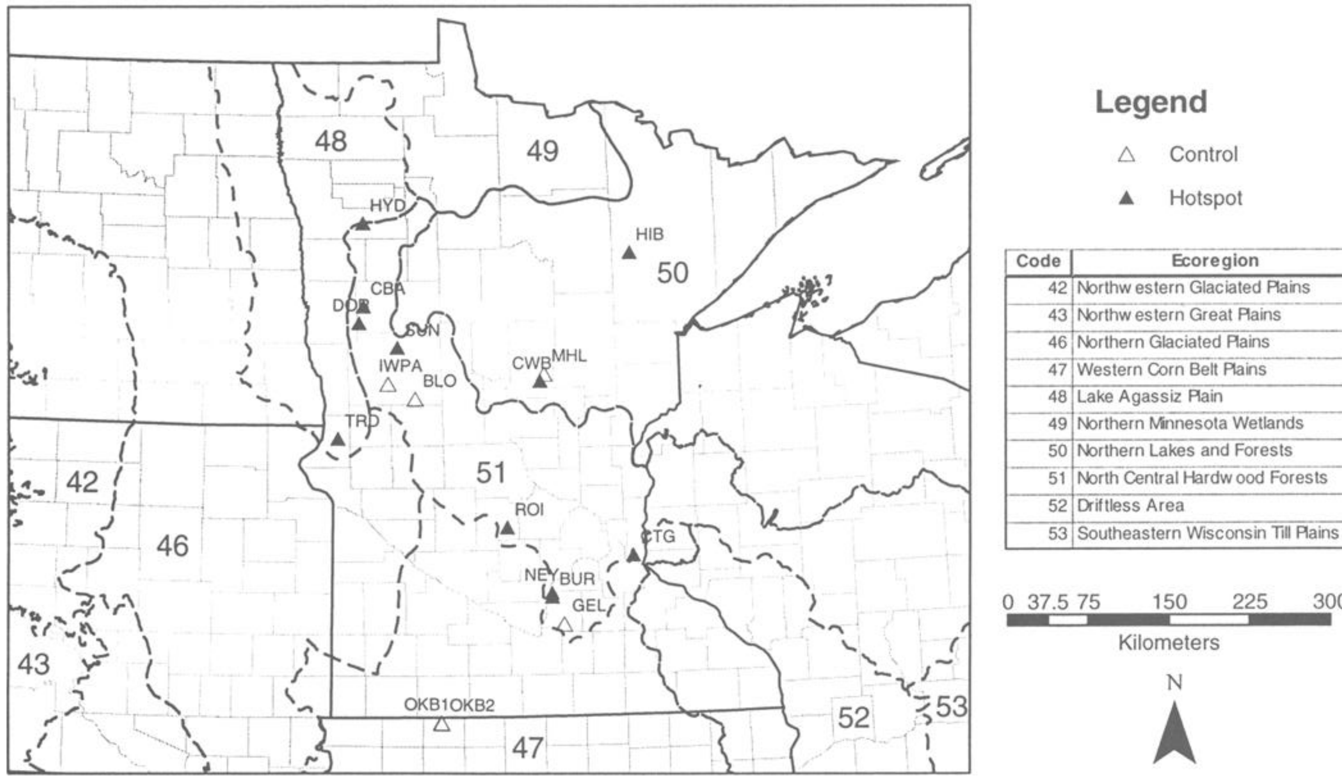

Figure 2 - *Location of Hotspot versus Reference (Control) Study Sites.*

Table 3 – *Basic Water Quality Data for the 17 Sites Sampled in the Minnesota Malformed Frog Survey. For Details on How These Data are Collected and Analyzed, see http://water.usgs.gov/owq. Bold Numbers Indicate the Two Highest and the Two Lowest Values Measured for Each Test.*

Station Name	Sp. Cond. (mS/cm)	DO (mg/L)	pH (units)	Alk (mg/L as $CaCO_3$)	Nitro Amn & Org Dis (mg/L as N)	Nitrogen Amm + Org Tot (mg/L as N)
BLO	237	**8.32**	8.36	108	0.864	**1.26**
BUR	291	7.49	7.71	132		
CBA	**738**	2.18	7.25	**311**	**0.47**	**0.71**
CTG	143	**7.95**	**9.37**	32	**0.672**	2.656
CWB	**45**	7.59	**9.00**	**21**		
DOR	425	**0.18**	**6.67**	204	**2.087**	**2.728**
GEL	316	0.72	7.05	142	1.712	**2.963**
HIB	255	5.06	8.92	69	0.941	1.272
HYD	458	6.79	**6.81**	188	1.602	2.074
IWPA	531	0.20	7.01	**266**	1.267	1.641
MHL	**30**	6.79	8.44	**12**		
NEY	336	6.95	7.42	141	1.757	1.958
OKB1	433	1.33	8.99	214	2.541E	2.46
OKB2	465	6.33	7.85	239	2.017	2.179
ROI	306	0.41	**6.81**	145	**2.588**	2.686
SUN	535	**0.15**	7.52	211	1.294	1.685
TRD	**627**	4.01	7.71	236		

Table 3 (continued)

Station Name	Phosphorus Total (mg/L as P)	Phosphorus D (mg/L as P)	Carbon Organic Dis. (DOC)	Ion Balance (% Difference)	Hardness (Total) mg/L as CaO3	Sodium D mg/L as Na	Potassium D mg/L as K
BLO	**0.0704**	0.0173	9.66	-5.3	110	2.19	6.49
BUR			11.50	2.93	160	1.2	1.11
CBA	0.168	0.1029	**5.47**	-1.67	**380**	6.43	2.64
CTG	0.239	0.025	**8.80**	**-11.11**	**24**	17.81	2.17
CWB		**< 0.01**	12.10			**0.8**	**0.81**
DOR	0.343	0.276	17.61	1.14	210	3.42	3.12
GEL	0.6	0.1069	17.99	5.38	160	4.98	2.38
HIB	**0.0769**	0.0292	14.96	-3.27	**79**	**19.58**	3.11
HYD	0.1718	0.0631	19.67	-2.29	210	2.73	4.35
IWPA	0.218	**0.1306**	18.14	**0.24**	**260**	1.48	5.28
MHL		**< 0.01**	9.40			**0.48**	**0.57**
NEY	0.208	0.1199	11.08	**8.87**	170	3.13	3.13
OKB1	**0.428**	0.363E	**29.23**	4.11	220	2.46	**10.85**
OKB2	0.241	0.1231	21.15	3.49	250	4.71	6.29
ROI	**1.533**	**1.078**	**33.66**	6.21	140	5.88	**11.15**
SUN	0.1233	0.0748	15.13	**0.04**	200	**30.34**	1.59
TRD		**< 0.01**	10.50			82	7

Table 3 (continued)

Station Name	Chloride D mg/L as Cl	Sulfate D mg/L as SO4	Calcium D mg/L as Ca	Magnesium D mg/L as Mg	Flouride D mg/L as F	Silica D mg/L as SIO2	Iron D (ug/L as Fe)
BLO	5.32	2.19	23.15	12.17	0.085E	5.41	42.35
BUR	3.76	12.73	37.72	15.05	0.25	1.01	17.29
CBA	4.59	**87.16**	**84.84**	40.2	0.192	23.28	62.74
CTG	19.5	3.5	6.39	1.86	<0.2	4.31	**15.59**
CWB			**5.3**	**1.4**		0.58	
DOR	5.79	4.07	42.31	25.16	**0.17**	19.49	229.3
GEL	10.72	1.5	37.61	16.38	**0.167**	15.25	295.76
HIB	**36.57**	1.92	18.68	7.81	0.095E	**0.28**	95.45
HYD	10.22	**25.87**	49.16	20.7	0.267	13.63	**12.93**
IWPA	**1.24**	0.48	**58.57**	**26.83**	0.110E	**67.13**	178.34
MHL			**4.3**	**0.89**		**0.45**	
NEY	8.58	0.92	33.12	21.37	0.207	13.65	**564.82**
OKB1	**3.03**	1.08	50.65	22.57	**0.528**	1.14	71.3
OKB2	5.96	1.1	49.43	**30.22**	**0.349**	3.3	259.18
ROI	4.5	**0.36**	39.77	10.51	0.087E	2.15	**495.97**
SUN	**41.29**	**0.47**	43.36	22.53	0.084E	**26.04**	125.49
TRD			25	14		19	

Table 3 (continued)

Station Name	Manganese D (ug/L as Mn)	Dissolved Solids (mg/L)
BLO	38.33	**132**
BUR	30.79	152
CBA	215.14	**436**
CTG	**<3**	**86**
CWB		
DOR	296.05	226
GEL	626.58	175
HIB	**4.42**	136
HYD	99.65	240
IWPA	**1251.45**	**322**
MHL		
NEY	**889.14**	170
OKB1	2.21E	220
OKB2	175.44	245
ROI	428.73	162
SUN	261.54	292
TRD		

and CTG (8; created, hotspot), CWB (8; natural, hotspot), MHL (8; natural, reference site) and ROI (8; natural, hotspot). Again, no patterns emerged.

Parasite Data

Several trends emerged from the parasite data (Table 4). Encysted echinostome metacercariae were found in the kidneys of animals from every site (Table 4). Other metacercariae, such as *Fibricola cratera* (Fib) and ochetosomatids (Mano), were found in frogs from a majority of wetlands. For the remaining parasite species there was strong site specificity; they could be present, often in high numbers, in amphibians from one site and completely absent in another. This was true of *Ribeiroia ondatrae*, which is important here because of the role it plays in causing amphibian malformations (Johnson et al. 1999, 2002).

Heavy *Ribeiroia* infections were indicative of hotspots (e.g., CTG, CWB, HIB) but lesser *Ribeiroia* infections might (e.g., BUR, GEL, NEY, ROI) or might not be associated with malformations (e.g., MHL; Table 4). Conversely, hotspots such as CBA, DOR, HYD, SUN and TRD showed no evidence of the presence of *Ribeiroia*. Among the four reference sites, the two Iowa wetlands (OKB1, OKB2) and IWPA also showed no evidence of the presence of *Ribeiroia*.

In 1999, four severely malformed mink frogs necropsied from CWB harbored a mean intensity of 110 *Ribeiroia* metacercariae (range 96-125). In 2000, 12 northern leopard frogs (10 malformed) from HIB were infected with *Ribeiroia* (mean intensity 155.5, range 51-266). Interestingly, the only two apparently normal frogs in the 2000 HIB sample had the two smallest *Ribeiroia* infections (51 and 52 metacercariae). Malformations at HIB in 2000 included cutaneous fusions, truncations, bony protuberances and soft tissue protuberances.

Where *Ribeiroia* occured, metacercariae were not found in every species of amphibian inhabiting the wetland. For example, in MHL (natural, reference site) *Ribeiroia* metacercariae were found in mink frogs (n = 4) but not in green frogs (n = 9); in ROI (natural, hotspot) *Ribeiroia* were found in leopard frogs (n = 11) but not in wood frogs (n = 4) or American toads (n = 1). This pattern may be due to sampling artifact. In general, species with longer larval stages have higher rates of *Ribeiroia* infection, and higher rates of infection among these species often correspond to higher numbers of amphibian species being infected.

At wetlands where *Ribeiroia* was present in every species of amphibian, infection rates varied across species. For example, at CWB (natural, hotspot) mink frogs (n = 9) averaged 35.4 *Ribeiroia* metacercariae per animal while the single green frog sampled had 12, a lower number than any mink frog. Similarly, at HIB (created, hotspot), wood frogs (n = 7) averaged 17.4 *Ribeiroia* metacercariae, leopard frogs (n = 11) averaged 7.9, and the one American toad had 3. An alternate explanation is to tie low American toad infection rates to their small body size (and therefore small target area for roaming cercariae). This may in part be true, but note the high *Ribeiroia* values for CTG (created, hotspot) American toads (17.1; n = 26), and HIB wood frogs (17.4; n = 7), both small-bodied anurans.

Perhaps the most surprising finding is the distribution of amphibians infected with *Ribeiroia* metacercariae (Fig. 3). Prior to this study, none of us had realized the strong tendency for *Ribeiroia* to occur predominantly in eastern Minnesota wetlands. This tendency extends to other sites in southeastern Minnesota and western Wisconsin (D. Sutherland, unpublished data). The majority of these sites are within the North Central Hardwood ecoregion although three occur in the Northern Lakes and Forest ecoregion. In our sample, no *Ribeiroia* sites occurred in grassland ecoregions.

Table 4 – *Parasites Dissected from Frogs Sampled During this Study. See Table 1 for Site Names. The First Number in each Column Represents Percent Occurrence. The Number in Parentheses is Mean Prevalence (Number of Parasites/Number of Animals Sampled).*

Site Code	Frog Species	Rib[1]	Fib[2]	Alaria	Globbie[3]	Mano[4]	Clino[5]	Thickie[6]	Kid Echin[7]	Gill Echin[8]	Masses	Choled
BLO	Rasy		20 (0.4)	80 (45)		40 (4.1)			90 (43.7)		20 (1)	
	Rapi		60 (2.8)		30 (0.3)	100 (62.8)			80 (16.1)	30 4.7	10 (0.6)	
BUR	Rapi	58 (3.08)	58 (5.5)		67 (4.7)	92 (3.2)			100 (55.7)			
CBA	Rapi		77 (58.4)	31 (9.3)	46 (2.2)	100 (118.9)			85 (13.0)	23 (4.2)	15 (1.0)	
CTG	Buam	100 (17.1)					4 (0.04)		42 (1.3)	4 (0.04)		
CWB	Rase	100 (35.4)		78 (62.8)	67 (1.9)	22 (0.2)	11 (0.1)		100 (241.4)		89 (36.1)	
	Racl	100 (12)			100 (1)				100 (275)			
DOR	Rapi		33 (1.9)			100 (37)			100 (25.7)	11 (22.2)		
GEL	Rapi	20 (3.4)	30 (9.4)	50 (60.9)	40 (1.2)	20 (0.6)	22 (2.0)		89 (40.1)		11 (1.5)	
	Buam		10 (1.2)	10 (0.4)								

Table 4 (continued)

Site Code	Frog Species	Rib[1]	Fib[2]	Alaria	Globbie[3]	Mano[4]	Clino[5]	Thickie[6]	Kid Echin[7]	Gill Echin[8]	Masses	Choled
IWPA	Rapi		9 (0.2)		55 (6.8)	73 (29.0)			82 (39)	9 (2.7)	18 (0.7)	
	Rasy		50 (7.2)	63 (6.9)	12 (0.4)	75 (9.2)			75 (86)		12 (0.7)	
MHL	Rase	25 (5)			100 (9)		50 (2)		50 (18.2)		100 (16.7)	
	Racl		11 (2.1)		44 (2.4)	11 (0.1)			44 (16)		100 (19.1)	
NEY	Rapi	36 (1)	27 (3.6)		91 (30.5)	18 (2.1)	27 (0.9)		91 (13.2)			
OKB1	Buam		44 (3.5)						11 (1.3)			
	Pstr		15 (11.6)						25 (1.2)			35 (2.6)
OKB2	Rapi		91 (112.4)	9 (10.6)	45 (43.8)	55 (146.9)	9 (0.1)		82 (114.4)		45 (8.1)	
ROI	Buam								100 (10)			
	Rasy			50 (9.2)					25 (4)			
	Rapi	36 (2.1)	18 (21)	18 (18.4)	64 (24.8)	64 (7.6)			91 (96.9)		36 (2.9)	

Table 4 (continued)

Site Code	Frog Species	Rib[1]	Fib[2]	Alaria	Globbie[3]	Mano[4]	Clino[5]	Thickie[6]	Kid Echin[7]	Gill Echin[8]	Masses	Choled
SUN	Rasy		89 (22.7)						33 (1.6)			
	Rapi		55 (62.2)	18 (6.4)	45 (5.4)	82 (6.5)	9 (0.3)	18 (1)	45 (2.7)	55 (10.5)		
TRD	Rapi		93 (67.2)			93 (27)			79 (6.8)			
	Buam		50 (3)									

[1] Rib = *Ribeiroia ondatrae*

[2] Fib = *Fibricola cratera*

[3] Globbies = a cohort of metacercariae that all possess globular excretory bladders; this group includes *Glypthelmins quieta*, *Auridistomum chelydrae* and numerous undetermined metacercariae

[4] Mano = ochetosomatid metacercariae; this group includes a number of trematodes that inhabit the lungs, pharynx and oral cavity of snakes (short for *Manodistomum* which is the worm Sessions and Ruth (1991) originally thought was the cause of malformations in *Hyla regilla* in California)

[5] Clino = *Clinostomum* is the yellow grub common in inshore fishes (but also common in frogs); adults are found in pharynx of herons, egrets and perhaps black-crowned night herons

[6] Thickie = *Apharyngiostrigea pipientis* possesses an extremely thick walled cyst

[7] Kid Echin = encysted echinostomes in kidney; Rebecca Cole (personal communication) believes there are several species involved

[8] Gill Echin = encysted echinostomes along cranial nerves exiting brain near gill resorption site

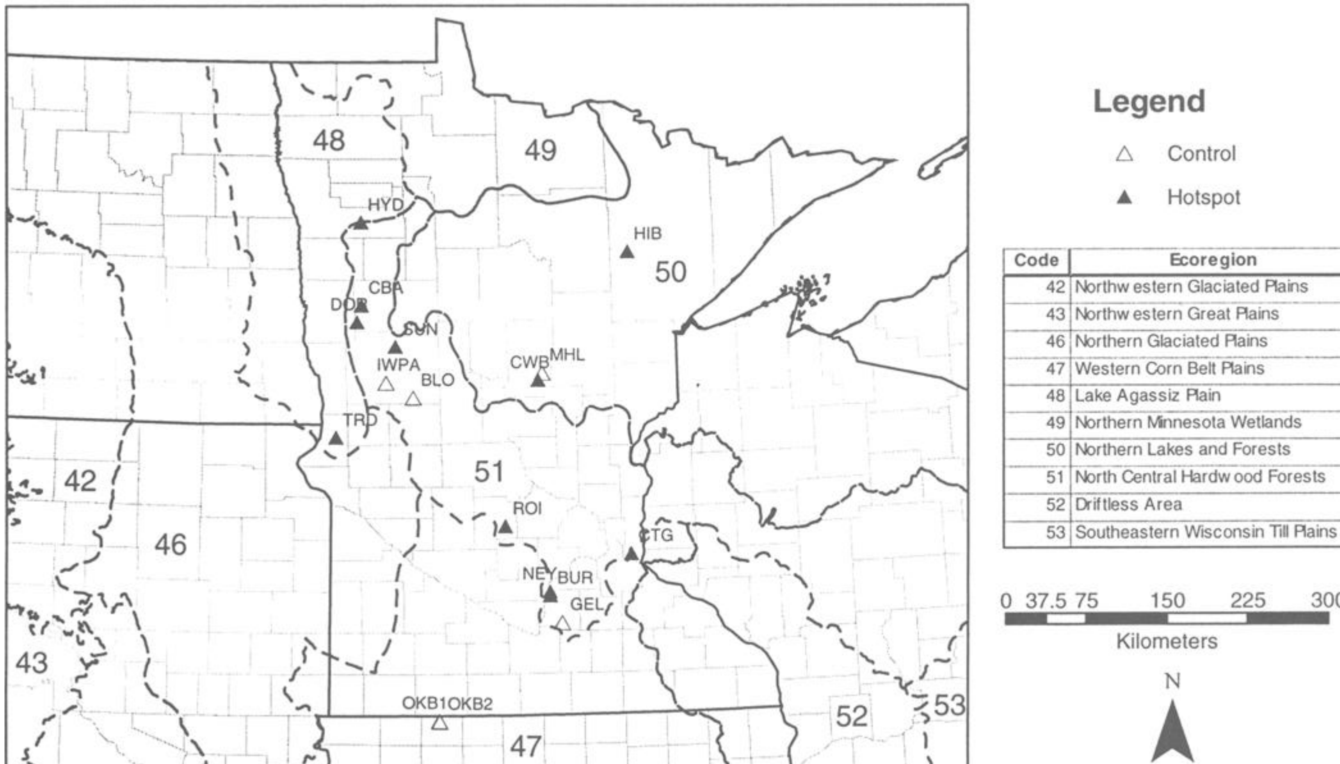

Figure 3 - *Location of Sites with the Trematode Parasite* Ribeiroia *Present Compared with Sites where the Parasite was Absent.*

Morphological Analysis

The 274 animals examined morphologically represented 6 species from 17 sites in Minnesota and Iowa. They form a subset of the 977 animals representing 16 species one of us (Lannoo) has examined from 73 sites in 13 states. For this analysis, we follow Meteyer et al. (2000, see also Johnson et al. 2001b, Ouellet 2000, Ouellet et al., 1997) and focus our attention on hindlimbs. Our malformation categories are summarized in Table 5 and include: amelia (completely missing limb, often times associated with missing pelvic elements); polymelia (duplications of limbs or limb elements); hemimelia (missing leg elements); micromelia (limb elements present but small); taumelia (or bony triangles, right angle, or nearly right angle bends in bones, see Gardiner and Hoppe 1999); limb hyperextension (rigid leg with immobility at hip, knee, and ankle joints; and spongiform bone (expansion of the cancellous bone at the distal tip of ectromeliac limbs or associated with taumelias; expansions are typically terminal, irregularly shaped, and only present on the affected limb).

We present our data by presence of malformation type by wetland (Table 5). Note that we do not present absence, nor do we present percentages, because neither our samples, nor samples from any other field studies conducted to date, have been done with sufficient rigor (i.e., drift fence studies) to determine that all malformed animals were sampled (and therefore that we can be truly sure malformation types were absent). Therefore, the only facts we can state are that animals with particular malformation types were found at particular wetlands (see Discussion for a more complete rationale).

The most common hindlimb malformation type (Ouellet et al. 1997, Meteyer et al. 2000) was ectromelia, followed by micromelia and the presence of spongiform bone. Limb hyperextension, amelia, and polymelia were the least common malformation types.

ROI, NEY, and CWB had the largest number of hindlimb malformation types, and aside from reference sites (GEL, IWPA, OKB1, OKB2), DOR, SUN, and TRD had the fewest (although HIB has produced large numbers of malformed frogs in past years, we collected no malformed animals in 2001 from HIB).

Discussion

Sites

Location — Malformed frog hotspots sampled in this study were not evenly, randomly, or even haphazardly distributed across Minnesota (Fig. 2); instead, most hotspots occurred in ecoregions with a mixed forest component. Hotspots do not tend to occur in ecoregions with a predominantly grassland or boreal forest component. Hotspots also tend to occur along the borders of ecoregions, not in their centers. Interestingly, humans also tend to live along the borders of ecoregions. Knowing this, several questions arise, including: does this pattern of hotspots reflect the true pattern of malformation locations? If it does, does this then reflect the direct influence of humans on the environment? If it does not, does it simply reflect the bias that human outdoor activities bring to the discovery of events in nature?

Origin — Hotspots can occur in natural or created wetlands; CWB is a natural site, HIB and NEY are created. It is important to realize that none of the 17 sites sampled were natural in any true sense. From among the reference sites, IWPA is surrounded by restored prairie, OKB1 and OKB2 are restored (although rich) wetlands, MHL has cabins along its north and east sides. Each of the other sites was associated with agriculture or housing developments. Some of the natural sites, such as ROI, HYD and

Table 5 – *Malformation Types Found at Each of Our Study Sites. See Table 1 for a Description of Sites, and the Text for a Description of the Malformation Types.*

Site name	Amelia	Polymelia	Ectromelia	Micromelia	Taumelia	Limb Hyper-extension	Spongiform Bone	Total
BLO					X			1
BUR				X			X	2
CBA								0
CTG	X		X		X		X	4
CWB		X	X		X	X	X	5
DOR			X	X			X	3
GEL								0
HIB								0
HYD	X		X	X			X	4
IWPA								0
MHL								0
NEY	X	X	X	X	X	X		6
OKB1								0
OKB2								0
ROI	X	X	X	X	X	X	X	7
SUN			X	X			X	3
TRD		X	X	X		X		4
Total	4	4	8	7	5	4	7	

CWB appear highly to moderately affected by human development and agricultural practices.

Water Quality — The battery of limnological tests we conducted did not reveal patterns of water quality, including acidification, underlying malformations. There can be several reasons for this, including that there are no patterns—that the chemistry of water does not influence the presence of malformations. This notion certainly contradicts some assumptions made during the course of the Minnesota Pollution Control Agency's investigation (Helgen et al. 1998, see Souder 2000); it also contradicts some results conducted from FETAX studies (Fort 1999a, 1999b) as well as other studies (Sparling 2000). A second reason may be that the problem is in the water, that it is chemical, but that it is separate from the more naturally-occurring chemicals sampled during limnological studies. Chemicals that would escape detection include pesticides, retinoids, and hormones, each of which have been implicated as causing amphibian malformations. We sampled for retinoids and certain hormones during the course of our study (analyses were not completed in time to make the manuscript deadline) but pesticides would not be detected in our sampling scheme. This explanation would be consistent with assumptions made by the Minnesota Pollution Control Agency and the results of Fort (1999a, 1999b).

A third reason for the lack of correspondence between water quality features and frog malformations may be that the problem is in the water but is biological rather than chemical. At least two lines of evidence (parasites, predation) suggest that biological causes can be important. The problem here is that biological causes cannot be generalizable to all hotspots. For example, failed predation can produce missing limbs, but the frequency of malformations at some sites in some years (> 60% of animals affected) and the absence of predators, or high densities of predators, at many sites argue against predation as a general cause. Similarly, the trematode parasite *Ribeiroia* has been shown experimentally to cause a wide range of malformation types in anurans (Johnson et al. 1999, 2001a) but these parasites do not occur at all malformation hotspots. Indeed, in one of our most intriguing results, *Ribeiroia* were only present in samples from the eastern half of Minnesota (Fig. 3).

A fourth reason for the lack of correspondence between water quality features and frog malformations could be that the problem is genetic. However, arguments for a genetic cause to malformations are undermined by three observations. First, hotspots are frequently found near sites that are not considered hotspots; close enough that individual frogs could, and probably do, migrate between normal sites and hotspots. Second, within hotspots, all or most species of frogs are affected. If there were a genetic cause underlying malformations, the observation that all species in one wetland are affected while no species in an adjacent wetland are affected would be highly improbable. Third, if there were a genetic component, one would expect that malformation types would sort by wetland types (that one wetland would produce, for example, amelia while another might produce polymelia; or that within wetlands, one species might produce amelia while another species would produce polymelia). So far, these patterns have not been observed. Additionally, Hoppe (in Volpe 2000) has experimental data that argue against genetic causes.

Morphological Signatures

This idea that morphology gives clues to malformation causes is implied by the title of the most recent paper on Minnesota frog malformations (Meteyer et al. 2000): "Hind limb malformations in free-living northern leopard frogs [*Rana pipiens*] from Maine, Minnesota, and Vermont suggest multiple etiologies." If true, this notion can be a powerful tool. Most malformed frogs are observed following metamorphosis. But hindlimb development occurs days, weeks, or months before this, depending on the species and local environmental conditions. This temporal disparity may be sufficient to allow whatever caused the malformations to leave, be washed out, degraded, or be diluted to the point where it is (they are) undetectable. It is for this reason that the concept of morphological signatures—malformation types that uniquely identify malformation causes—has been attractive, because it allows causes to be inferred from morphology. Less desirable but still useful is the idea that certain malformation types may allow the exclusion of potential causes (Lannoo 2000).

For example, early in the investigation of U.S. amphibian malformations, "bony triangles" (taumelia) were seen as indicative of retinoic acid involvement (Gardiner and Hoppe 1999, see also Souder 2000). However the demonstration by Johnson et al. (1999) that *Ribeiroia odonatrae* metacercariae can also cause this malformation type (perhaps through the secretion of chemicals with retinoid properties) forced us to abandon the idea that bony triangles were unique morphological signatures for water-born exposure to retinoid, or retinoid-like compounds.

In another example, Ankeley et al. (1998) showed that exposure of tadpoles to UV-B radiation produces bilaterally symmetrical ectromelias. However, bilaterally symmetrical ectromelias are rarely found in nature (Souder 2000, Lannoo, unpublished data; see also Meteyer et al. 2000, Johnson et al. 2002). Further, when bilaterally symmetrical ectromelias are found, in our experience these malformations are associated with spongiform bony expansions. None of the animals produced by Ankeley et al.'s (1998) experiments exhibited spongiform bone. What we can say from Ankeley et al.'s data is that there is no evidence that unilateral malformations, or malformations with spongiform bone associations, are caused solely by overexposure to UV-B.

Hindlimb malformations may also be produced by failed predation attempts (see Lannoo 2000 for a full discussion of this possibility). However, 74% of the ectromelias in animals we sampled showed spongiform bone. Our results with experimental amputations (Lannoo, in preparation) show that (as with UV-B exposure) laboratory amputations also fail to produce spongiform bone. However, we cannot exclude the possibility that spongiform bone is produced by conditions in the wetland, perhaps by other animals aggravating the wound; conditions that are not duplicated in more controlled laboratory settings). What we can say is that there is no experimental evidence that ectromelias associated with spongiform bone are caused by failed predation attempts.

Johnson et al. (1999, 2001a, 2002) reported a range of malformations induced experimentally by the trematode parasite *Ribeiroia* and from field-collected animals containing encysted *Ribeiroia* metacercariae throughout the western USA. We radiographed 125 of these frogs. Eight species are represented and there appears to be no morphological signature for *Ribeiroia* infections. Hemimely, ectromely, and polymely were common malformations in 104 *Ribeiroia* infected animals. These malformation types were also common in 21 field-collected animals from sites with no evidence of parasites. Spongiform bone was not exhibited in Johnson et al.'s (1999) experimental animals and tends not be associated with *Ribeiroia* infections. We continue to explore this dissociation but have drawn no conclusions. Therefore, we determine that in contrast to the title (but, interestingly, not the text) of Meteyer et al. (2000) there is little evidence from Minnesota frogs that causes of malformations can be inferred from morphological signatures.

At the national level, however, three sites have produced malformation types that are so bizarre or unique that we suspect their causes to be unique. In Trempealeau County, Wisconsin, Sutherland sampled 27 newly metamorphosed, malformed green frogs (*Rana clamitans*) from a site that included 16 unilateral hindlimb ectromelic animals, 5 bilateral hindlimb ectromelic animals, 4 unilateral hindlimb ectromelic animals, one hindlimb polydactly, and one animal with a unilateral taumelia. One animal had a unilateral hindlimb hemimely and a contralateral ectromely. This ectromely and one other ectromely were associated with missing pelvic elements, including iliums. Sixteen of the hemimelic animals (all but two cases) were associated with bony expansions. But unlike the spongiform bony expansions described here (above) and seen in most circumstances, expansions also occurred in the contralateral, normal (gross appearance) limbs in two hemimelic animals with bony expansions from this site (Fig. 4a). Also unlike spongiform bone, these expansions could be either terminally or subterminally positioned and were symmetrical. Subterminal expansions were not associated with the site of penetrating arteries. In four cases cancellous bone was clearly expanded in conjunction with compact bone. But in ten cases compact bone, not cancellous bone, was differentially expanded. Therefore, this Wisconsin site was unique in producing regularly-shaped, subterminal or terminal bone expansions characterized by abnormal compact bone growth. Furthermore, these expansions could occur in both ectromelic and the contralateral normal limbs. It is worth noting that in the four years of studying this site, not one metacercaria of *Ribeiroia* has been found.

One of Facemire's Switzerland County, Indiana, sites contained a sample of 13 late tadpole stage or newly metamorphosed bullfrogs (*Rana catesbeiana*) and demonstrated several types of malformations. Four animals had edematous swellings, which we term hygromas (subcutaneous, serous fluid-filled swellings), surrounding their lower limbs or associated with their ventral pelvic regions (Fig. 4b). Two of these animals had bilaterally symmetrical, serially arranged hygromas, with separate edemas occurring in femoral and tibiofibular segments—that is, when these hygromas were associated with a limb they encompassed a limb segment, extending from joint to joint, for example hip to knee, knee to ankle. One of these animals had a pair of duplicated hindlimbs. Other malformations occurring in this sample included a malformed mandible, a kinked tail, and a hindlimb ectromely. Radiographs of the proximal femur of this limb showed no spongiform bony expansion.

One of Johnson and Lunde's Santa Clara County, California sites contains bullfrogs with *Ribeiroia* infections. These animals are characterized by having long bones that bend at the site where nutrient arteries penetrate (Fig. 4c). This is true for all long bones in a hindlimb, true bilaterally, and true for forelimbs bilaterally.

Can We Identify with Assurance Specific Causes of Malformations at Particular Locations?

Whether they are considered hotspots or not, sites with *Ribeiroia* are likely to support malformed animals, and some percentage of these malformations are no doubt caused by the metacercariae. In our sample, these *Ribeiroia* positive sites are all located in the eastern half of Minnesota (Fig. 3) and include BUR, CTG, CWB, GEL, HIB, MHL, NEY, ROI (Table 4).

In hotspots where *Ribeiroia* infections were absent, this parasite cannot be the cause of observed malformations. These sites include DOR, HYD and TRD. In one of these sites (TRD), macrophyte beds that provide habitat for planorbid snail hosts were undeveloped at the time of our sampling, and in three years of sampling this site Hoppe (unpublished data) has failed to find snails of any species. In the absence of host habitat, hosts, and metacercariae it is difficult to argue for parasites as a cause for malformations. It is also unlikely that metacercariae have died and been "cleared" from animals, thus escaping detection. As amphibians metamorphose and proceed from being aquatic organisms to becoming terrestrial, their parasitic fauna changes—they lose some aquatic-associated species and gain terrestrial-associated species such as the lung fluke, *Hematoloechus* sp. that the frog acquires from ingesting infected dragonflies (Sutherland

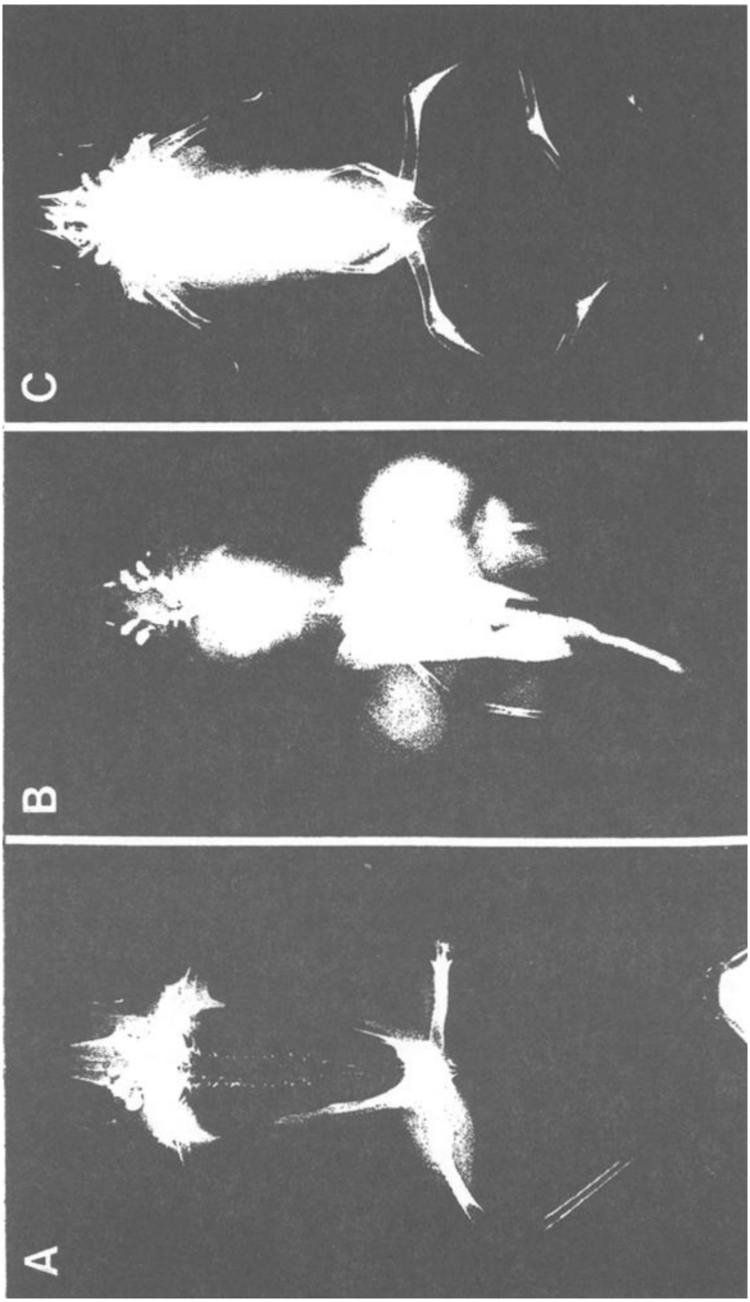

Figure 4 - *Malformation Types Characteristic of Particular Wetlands. A) A Northern Leopard Frog from Trempealeau County, Wisconsin. B) An American Bullfrog from Switzerland County, Indiana. C) An American Bullfrog from Santa Clara County, California. See Text for Details of these Malformations.*

and Kapfer, unpublished data). Therefore, the absence of *Ribeiroia* from an aquatic-associated parasitic fauna indicates a lack of infection, not infection with subsequent clearing (also consider the time from hindlimb development to metamorphosis in *Bufo* species can be as short as one week). On the other hand, if sufficient time has occurred to develop a terrestrial parasitic fauna, we can reasonably expect that evidence of some aquatic infections will wane.

At this point, two questions come to mind (which may have related answers). The first is: In sites with *Ribeiroia*, what environmental factors affect their abundance (intensity) such that, for example, mink frogs in CWB have an average of 35.4 metacercariae/animal while mink frogs in nearby MHL have an average of 5.0—a 7-fold difference? There are many differences between CWB and MHL including size (CWB is much smaller), biology (MHL has a sphagnum bog), and land use (MHL has cabins; CWB hosts dairy cattle that create an erosion problem along a portion of the shoreline). Environmental conditions that produce differences in *Ribeiroia* infection intensity may be natural or not. If not, it is useful to consider that eutrophication associated with cattle use will provide more and richer plant life (phytoplankton and macrophyte beds) when compared to less abused wetlands, and this plant life provides food and habitat for the planorbid snail hosts of *Ribeiroia* (Johnson and Lunde 2002). It is also useful to consider that with modern farming techniques, including feed additives, cattle feces and urine contain many more substances than the natural byproducts of ingestion and metabolism (as we write this, pollution by such additives is being perceived as an unrecognized, but large problem in U.S. waterways - see http://toxics.usgs.gov/regional/emc.html). We also await the analyses of data we collected on the presence of hormone and retinoid compounds.

The second question is: What effect does the presence of *Ribeiroia* have on our consideration of other potential causes of malformations? In part this is a question with a social component. There has been so much contention surrounding the discovery of causes of malformed frogs that finding one cause has tended to be interpreted as excluding other causes (see Souder 2000). This is a false assumption. While CWB contains *Ribeiroia*, because this wetland was littered with frog and fish carcasses this trematode cannot be the only problem at CWB. *Ribeiroia* is not known to cause mass die-offs in normally appearing tadpoles, adult frogs, or fishes. Further, the absence of *Ribeiroia* in the western portion of Minnesota indicates some factor other than parasites must be causing malformations, and it is not clear why this factor should partition itself in an east-west pattern mutually exclusive of the distribution of *Ribeiroia*.

Conclusions, With Recommendations for Land Owners and Managers, and a Larger View

Amphibian malformations have several causes and in the best of all possible worlds each hotspot would be carefully examined, the cause determined, and the source of the cause eliminated. This process is not only costly, but time consuming, and given the current funding crises experienced by governments at all levels, it is also not likely to happen anytime soon. Instead, it might be better to recognize that hotspots tend to be altered wetlands. These alterations grade from what appears to be benign causes (simply being created and perhaps not having the buffering capacity of more mature systems; these sites include TRD and perhaps BUR, CBA, NEY), through what is perhaps simple eutrophication (which would lead to increased plant growth, increased snail populations, and increased *Ribeiroia* levels [Johnson and Lunde 2002]; these sites include HIB, perhaps DOR), through eutrophication with suspected additional chemical inputs (CWB, ROI, perhaps CTG, HYD, SUN). It is this latter category that should be of most concern to humans.

Recognizing that hotspots are altered wetlands, probably the quickest and least expensive way to reduce malformations is to recognize the nature of the alterations and take steps to eliminate them. For example, is part of the eutrophication and suspected chemical input to ROI due to leaky septic systems? This is easily tested and if so, these waste control systems should be upgraded. Is part of the eutrophication and suspected chemical input to CWB due to the utilization of this wetland by cattle? This is an especially instructive question, because while the debate on malformation causes has tended to focus on proximate causes (for example retinoids versus parasites; see Souder 2000) the fact is that both could be present, and both could be caused by a single factor: cattle usage (nutrients produce eutrophication which produces snails which produce trematodes; retinoids enter the water as a non-digested component of feed additives). In either case, parasites or retinoids, the solution at CWB might be to remove the cattle.

Such solutions, however, are rarely simple because they involve value systems. For example, at CWB, does the right of a homeowner to live without health concerns caused by environmental degradation trump a farmer's need to water cattle? At ROI do health concerns trump the cost of upgrading septic systems? Does a state's right to manage public property trump the cost of private individuals practicing agricultural erosion control? Value systems then, in our view, become a central issue in solving the malformed frog problem, and as a society, we have found these decisions difficult to make.

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References

Ankeley, G. T., Tietge, J. E., DeFoe, D. L., Hjensen, K. M., Holcombe, G. W., Durhan, E. J., and Diamond. A., 1998, "Effects of Ultraviolet Light and Methoprene on Survival and Development of *Rana pipiens*," *Environmental Toxicology and Chemistry*, Vol. 17, pp. 2530-2542.

Antweiler, R. C., Patton, C. J., and Taylor, H. E., 1993, "Automated, Colorimetric Methods for the Determination of Nitrate, Nitrite, Ammonium and Phosphate Ions in Natural Water Samples," U.S. Geological Survey Open-File Report No. 93-638, Washington, D.C.

Blaustein, A. R., Kiesecker, J. M., Chivers, D. P., and Anthony, R. G., 1997, "Ambient UV-B Radiation Causes Deformities in Amphibian Embryos," *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 94, pp. 13735–13737.

Brinton, T. I., Antweiler, and Taylor, H. E., 1996, "Method for the Determination of Dissolved Chloride, Nitrate, and Sulfate in Natural Water Using Ion Chromatography," U.S. Geological Survey Open-File Report No. 95-426A, Washington, D.C.

Cowardin, L. M., Carter, V., Golet, F.C., and LaRoe, E. T., 1979, *Classification of Wetlands and Deepwater habitats of the United States*, U.S. Fish and Wildlife Service, FWS/OBS/79/31, Washington, D.C.

Fort, D. J., Propst, T. L., Stover, E. L., Helgen, J. C., Levey, R. B., Gallagher, K., and Burkhart, J. B., 1999a, "Effects of Pond Water, Sediment, and Sediment Extracts from Minnesota and Vermont, USA, on Early Development and Metamorphosis of *Xenopus*," *Environmental Toxicology and Chemistry*, Vol. 18, pp. 2305-2315.

Fort, D. J., Propst, T. L., Stover, E. L., Helgen, J. C., Levey, R. B., Gallagher, K., and Burkhart, J. B., 1999b, "Progress Towards Identifying Causes of Maldevelopment Induced in *Xenopus* by Pond Water and Sediment Extracts," *Environmental Toxicology and Chemistry*, Vol. 18, pp. 2316-2324.

Gardiner, D. M., and Hoppe, D. M., 1999, "Environmentally Induced Limb Malformations in Mink Frogs (*Rana septentrionalis*)," *Journal of Experimental Zoology*, Vol. 284, pp. 207-216.

Helgen, J., McKinnell, R. G., and Gernes, M. C., 1998. "Investigation of Malformed Northern Leopard Frogs in Minnesota," *Status and Conservation of Midwestern Amphibians*, M. J. Lannoo, Ed., University of Iowa Press, Iowa City, IA.

Johnson, P. T. J., Lunde, K. B., Ritchie, E. G., and Launer, A. E., 1999, "The Effect of Trematode Infection on Amphibian Limb Development and Survivorship," *Science*, Vol. 284, pp. 802-804.

Johnson, P. T .J., Lunde, K. B., Ritchie, E. G., Reaser, J. K., and Launer, A.E., 2001b, "Morphological Abnormality Patterns in a California Amphibian Community," *Herpetologica*, Vol. 57, pp. 336-352.

Johnson, P. T. J., Lunde, K. B., Haight, R. W., Bowerman, J., and Blaustein, A. R., 2001a, "*Ribeiroia ondatrae* (Trematoda: Digenea) Infection Induces Severe Limb Malformations in Western Toads (*Bufo boreas*)," *Canadian Journal of Zoology*, Vol. 79, pp. 370-379.

Johnson, P. T. J., Lunde, K. B., Thurman, E. M., Ritchie, E. G., Wray, S. W., Sutherland, D. R., Kapfer, J. M., Frest, T. J., Bowerman, J., and Blaustein, A.R., 2002, "Parasite Infection Linked to Amphibian Malformations in the Western United States," *Ecological Monographs* 72:151-168.

Johnson, P. T. J. and Lunde, K. B., 2002, "Trematode Parasites and Amphibian Limb Malformations in the Western US: Are They a Concern?" *Status and Conservation of U.S. Amphibians. Volume 1: Conservation Essays*, M. J. Lannoo, Ed., University of California Press, Berkeley, CA.

Lannoo, M. J., 2000. "Conclusions Drawn from the Malformity and Disease Session," *Journal of the Iowa Academy of Science*, Volume 107, pp. 212-216.

McKinnell, R.G., Hoppe, D.M., and McKinnell, B.K, 2003, "Burnsi and Kandiyohi Pigment Pattern Morphs of the Northern Leopard Frog (*Rana pipiens*): Distribution and Prevalence," *Status and Conservation of U.S. Amphibians. Volume 1: Conservation Essays*, M. J. Lannoo, Ed., University of California Press, Berkeley, CA.

Merrell, D.J., 1965, "The Distribution of the Dominant Burnsi Gene in the Leopard Frog, *Rana pipiens*," *Evolution*, Vol. 19, pp. 69-85.

Meteyer, C. U., Loeffler, I. K., Fallon, J. F., Converse, K. A., Green, E., Helgen, J. C., Kersten, S., Levey, R., Eaton-Poole, L., and Burkhart, J.G., 2000. "Hind Limb Malformations in Free-living Northern Leopard Frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont Suggest Multiple Etiologies," *Teratology*, Vol. 62, pp.151-171.

Mitko, K. and Bebek, M., 2000, "Determination of Major Elements in Saline Water Samples Using a Dual-view ICP-OES," *Atomic Spectroscopy*, Volume 21, pp. 77-85.

NARCAM (North American Reporting Center for Amphibian Malformations), 2001, Northern Prairie Wildlife Research Center Home Page: http://www.npwrc.usgs.gov/narcam. Jamestown, ND.

Ouellet, M., Bonin, J., Rodrigue, J., DesGranges, J. L., and Lair, S., 1997, "Hindlimb Deformities (Ectromelia, Ectrodactyly) in Free-living Anurans from Agricultural Habitats," *Journal of Wildlife Diseases*, Vol. 33, pp. 95–104.

Ouellet, M., 2000, "Amphibian Deformities: Current State of Knowledge," *Ecotoxicology of Amphibians and Reptiles*, D. W. Sparling, G. Linder, and C. A. Bishop, Eds., SETAC Press, Pensacola, FL.

Rowe, C. L., Kinney, O. M., and Congdon, J. D., 1998, "Oral Deformities in Tadpoles of the Bullfrog (*Rana catesbeiana*) Caused by Conditions in a Polluted Habitat," *Copeia*, Vol. 1998, pp. 244–246.

Souder, W. 2000, *A Plague of Frogs: The Horrifying True Story*, Hyperion Press, NY.

Sparling, D. W., 2000, "Field Evidence for Linking Altosid Applications with Increased Amphibian Deformities in Southern Leopard Frogs," *Journal of the Iowa Academy of Science*, Volume 107, pp. 90-91.

Steinbeck, J. and Ricketts, E.F., 1941. *The Sea of Cortez*, Viking Press, Inc. NY.

U.S. Geological Survey, 2001, *Malformed Frogs in Minnesota: an Update*, Fact Sheet Number 043-012001, U.S. Department of the Interior, U.S. Geological Survey Mounds View, MN.

Volpe, E. P., 2000, *Understanding Evolution*, McGraw-Hill, NY.

Wershaw, R. L., Fishman, M. J., Grabbe, R. R., and Lowe, L. E., 1983, "Methods for the determination of Organic Substances in Water and Fluvial Sediments," U.S. Geological Survey Techniques of Water-Resources Investigations, Book 5, Chapter A3, Washington, D.C.