Ribeiroia ondatrae (Trematoda: Digenea) infection induces severe limb malformations in western toads (*Bufo boreas*)

Pieter T.J. Johnson, Kevin B. Lunde, Ryan W. Haight, Jay Bowerman, and Andrew R. Blaustein

Abstract: Widespread reports of malformed amphibians in North America have prompted investigations into the cause(s) and implications of the phenomenon. Recently, a trematode parasite (*Ribeiroia ondatrae*) was identified as the probable cause of hind-limb malformations in Pacific treefrogs (*Hyla regilla*) from California. We exposed a second anuran species, the western toad (*Bufo boreas*), to specific levels of *R. ondatrae* infection. In a dose-dependent manner, *R. ondatrae* infection induced high frequencies (40–85%) of severe limb malformations in surviving toads. Survivorship declined significantly with increasing parasite exposure, falling to 42% in the heaviest treatment. Larvae in control treatments exhibited normal development and low mortality levels. In contrast to previous experiments with *R. ondatrae* infection in treefrogs, cutaneous fusion was the predominant malformation among infected toads in all treatments. Infection also caused polymely (extra limbs; fore and hind), ectromely (missing limbs), polydactyly (extra digits), and a variety of additional limb malformations. Taken together, these results demonstrate that (*i*) the teratogenic effects of *R. ondatrae* are not limited to treefrogs, (*ii*) the spectrum of *R. ondatrae*-induced malformations is not confined to the hind limbs, and (*iii*) the frequency and composition of malformations resulting from infection may vary among amphibian species. Finally, we review historical reports of limb abnormalities in the genus *Bufo* and discuss established and proposed causative agents, with emphasis on trematode infection and predation.

Résumé : Les malformations chez les amphibiens sont de plus en plus signalées en Amérique du Nord; nous avons cherché à déterminer les causes et les conséquences de ce phénomène. Récemment, le trématode parasite (*Ribeiroia ondatrae*) a été reconnu comme la cause probable des malformations des membres postérieurs chez la Rainette du Pacifique (*Hyla regilla*) en Californie. Nous avons soumis une seconde espèce d'anoure, le Crapaud de l'ouest (*Bufo boreas*), à des infections de gravités diverses de *R. ondatrae*. Les infections de *R. ondatrae* entraînent des fréquences élevées (40–85 %) de malformations sérieuses des membres en fonction de la dose administrée chez les crapauds survivants. La survie diminue significativement à mesure qu'augmente l'exposition aux parasites, jusqu'à 42 % à la suite des traitements les plus sévères. Chez les larves des groupes témoins, le développement est normal et la mortalité est faible. Contrairement aux résultats d'expériences antérieures sur les rainettes, ici c'est la fusion cutanée qui constitue la principale malformation chez les crapauds infectés, à la suite de tous les traitements. Les infections sont aussi à l'origine de polymélies (membres surnuméraires, antérieurs et postérieurs), d'ectromélies (membres manquants), de polydactilies (doigts surnuméraires) et d'autres malformations des membres. Considérés dans leur ensemble, ces résultats démontrent que (*i*) les effets tératogènes de *R. ondatrae* ne sont pas restreints aux rainettes, (*ii*) l'éventail des malformations attribuables à *R. ondatrae* n'affecte pas que les membres postérieurs et (*iii*) la fréquence et la nature des malformations dues aux infections peuvent varier chez les différentes espèces d'amphibiens. Nous avons passé en revue tous les rapports historiques sur les malformations des membres chez *Bufo* et nous examinons ici les agents putatifs reconnus et supposés, en insistant sur les infections causées par les trématodes et sur la prédation.

[Traduit par la Rédaction]

Introduction

Severe malformations, including missing, malformed, and supernumerary limbs, have been reported recently in dozens of amphibian species from diverse aquatic habitats across North America (Kaiser 1997; Ouellet et al. 1997; Helgen et al. 1998; Johnson et al.[2]). The frequency of abnormalities in affected populations often exceeds the expected base-line

P.T.J. Johnson,[1] K.B. Lunde, and R.W. Haight. The Roberts Environmental Center, Claremont McKenna College, W.M. Keck Science Center, 925 North Mills Avenue, Claremont, CA 91711-5916, U.S.A.
J. Bowerman. Sunriver Nature Center, Box 3533, Sunriver, OR 97707, U.S.A.
A.R. Blaustein. Department of Zoology, Oregon State University, Corvallis, OR 97331-2914, U.S.A.

[1]Corresponding author (e-mail: pieter_johnson@mckenna.edu).

[2]P.T.J. Johnson, K.B. Lunde, E.M. Thurman, E.G. Ritchie, S.W. Wray, D.R. Sutherland, J.M. Kapfer, T.J. Frest, J. Bowerman, and A.R. Blaustein. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. Submitted for publication.

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frequency, 0–5% (Martof 1956; Dubois 1979; Meyer-Rochow and Asashima 1988; Tyler 1998), generating concern about the impact of malformations on population viability. Malformations are suspected to be deleterious to survival and are infrequently reported among adult amphibians (Sessions and Ruth 1990; Veith and Viertel 1993; Ouellet et al. 1997; Johnson et al. 2001). Although malformations have not been associated with widespread amphibian population declines, they could exacerbate declines in already threatened populations, particularly if such malformations have recently increased in frequency, prevalence, or severity (Hoppe 2000).

Potential causes of limb malformations reported in amphibians include biocide contamination (Ouellet et al. 1997; Burkhart et al. 1998; Fort et al. 1999; but see Tietge et al. 2000), retinoid mimics (Gardiner and Hoppe 1999), predation (Bohl 1997a), UV-B radiation (Ankley et al. 1998), and trematode infection (Sessions and Ruth 1990; Sessions et al. 1999; Johnson et al. 1999, 2001; Johnson et al.[2]). For the majority of affected populations, however, proximate causes of the malformations have not been identified. Johnson et al. (1999), in a study of Pacific treefrogs (*Hyla regilla*) from California, reported a positive correlation between field sites with high frequencies of limb malformations and infection with a trematode parasite, *Ribeiroia ondatrae*. Experimental exposures of laboratory-raised Pacific treefrog larvae to *R. ondatrae* cercariae isolated from naturally infected snails (*Planorbella tenuis*) induced high frequencies of hind-limb malformations strikingly similar to those observed at study sites (Johnson et al. 1999). These malformations included extra limbs and digits, missing limbs and digits, cutaneous fusion, and taumely (abnormalities are described in Table 1). Abnormalities were usually unilateral and, when bilateral, asymmetrical. Johnson et al. (1999, 2001) suggested that these malformations may benefit *R. ondatrae* by increasing the susceptibility of infected frogs to predation by final hosts (see also Sessions and Ruth 1990). Parasites in the genus *Ribeiroia* cannot complete their life cycle and reproduce sexually unless the amphibian host is ingested by an appropriate avian or mammalian host (Beaver 1939; Basch and Sturrock 1969).

Although *R. ondatrae* is known to cause limb malformations in Pacific treefrogs, its impact on other amphibian species has not been examined. In the current study we exposed western toad (*Bufo boreas*) larvae to specific quantities of *R. ondatrae* cercariae. The western toad, once an abundant anuran in the western U.S.A., has suffered substantial population declines and local extinctions over the last 50 years (Carey 1993; Blaustein et al. 1994; Stebbins and Cohen 1995; Drost and Fellers 1996). We selected it as an experimental species because it exhibits rapid larval development, is phylogenetically distant from the previously studied Pacific treefrog, and is native to North America. Additionally, abnormal western toads infected with *R. ondatrae* metacercariae have been recorded recently from several sites in the Pacific Northwest (Johnson et al. 1999; Johnson et al.[2]). Our specific objectives were (*i*) to determine the effects of specific *R. ondatrae* exposures on malformation frequency and survivorship in western toads, (*ii*) to identify the types of malformations resulting from infection and compare them with those observed in experiments with Pacific treefrogs, and (*iii*) to evaluate *R. ondatrae* infection and other known causes of limb abnormalities in natural toad populations.

Materials and methods

We collected western toad embryos from field sites in the eastern Cascade Mountains of Oregon and immediately transported them to laboratory facilities. Individual larvae were isolated in plastic containers filled with 1 L of commercial spring water and maintained at 23°C. A 1:1 feed mixture of Tetramin® tropical fish food and *Spirulina* vegetable supplement was added to containers every second day for the duration of the experiment. We randomly assigned 220 larvae to one of four treatments that differed in level of exposure to *R. ondatrae*: control (0 cercariae), light (16 cercariae), intermediate (32 cercariae), and heavy (48 cercariae). Sample sizes were as follows: 60 larvae in the control, 55 in each of the light and intermediate treatments, and 50 in the heavy treatment. Beginning at approximately stage 27 (Gosner 1960), we exposed larvae to one-fourth of their intended parasite load every third day for 10 days (days 1, 3, 7, and 10). All infections were completed at or prior to stage 30. We obtained *R. ondatrae* cercariae from infected snails (*P. tenuis*) collected from field sites in northern California (described in Johnson et al. 2001). Snails were placed individually in 50-mL vials of spring water and allowed to shed parasites between 18:00 and 01:00. Cercariae were immediately isolated, counted using a stereodissecting microscope, and administered at the appropriate dosage to western toad larvae in 100-mL specimen cups. Exposures to parasite cercariae lasted approximately 120 min. Larvae in the control treatment were isolated in specimen cups containing only spring water. Before returning larvae to 1-L plastic containers, a subsample of specimen cups in each parasite treatment were inspected to ensure that all cercariae had penetrated the larvae (i.e., no cercariae remained free-swimming). Following the final parasite exposure on day 10, toad larvae were allowed to develop until metamorphosis (stage 44), at which time they were euthanized in MS 222, fixed in 10% phosphate-buffered formalin, and stored in 70% ethanol. All animals were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care (1993).

Specimens were measured and scored for abnormalities using a stereodissecting microscope. The terminology and classification system for abnormalities used here was described by Johnson et al. (2001). The frequency of abnormalities within a treatment was calculated as the percentage of malformed individuals among animals that survived to metamorphosis. We described the composition of abnormalities by evaluating the relative frequency of each abnormality type within a given treatment (Table 1). The severity of abnormalities was calculated as the mean number of discrete abnormalities per abnormal toad in a treatment (e.g., an extra right hind limb and a missing left forearm) (Johnson et al. 2001).

Results

Ribeiroia ondatrae infection induced severe limb malformations in 67% of the exposed larvae that survived to metamorphosis (*n* = 85). The frequency of malformed individuals within a treatment showed a significant functional response to the level of *R. ondatrae* exposure, increasing directly with parasite dose (logistic regression, χ^2 = 80.51, df = 3, *P* < 0.0001; Fig. 1). Eighty-six percent of the emerging toads in the heavy treatment exhibited one or more severe malformations (Table 1, Fig. 2). Survivorship in all treatment groups declined sharply with increasing parasite exposure and fell below 45% in the heaviest treatment (logistic regression,

Table 1. Malformation types and their relative frequencies from field and experimental studies of western toads (*Bufo boreas*).

Abnormality type	Control (0/47)	Light treatment (13/33)	Intermediate treatment (26/31)	Heavy treatment (18/21)	Combined (57/85)	Field (129/2475)
Cephalic and axial						
Anophthalmy (missing eye)	0	0	0	0	0	2.1
Mandibular hypoplasia (malformed jaw)	0	0	0	0	0	0.7
Open wound	0	0	0	0	0	2.8
Other	0	0	0	0	0	0.7
Forelimb						
Ectrodactyly (missing digit)	0	0	1.6	0	0.8	6.9
Polydactyly (extra digit)	0	0	0	0	0	0
Apody (missing hand)	0	0	0	0	0	1.4
Hemimely (partially missing limb)	0	0	1.6	0	0.8	0.7
Ectromely (missing limb)	0	8.7	6.6	0	4.7	1.4
Polymely (extra limb)	0	0	3.3	0	1.6	0
Other malformations[a]	0	0	0	0	0	1.4
Hind limb						
Ectrodactyly (missing digit)	0	0	3.3	2.2	2.3	23.6
Polydactyly (extra digit)	0	13.0	14.8	11.1	13.2	2.8
Apody (missing foot)	0	0	0	2.2	0.8	9.0
Polypody (extra foot)	0	0	4.9	4.4	3.9	0
Hemimely (partially missing limb)	0	0	0	0	0	18.1
Ectromely (missing limb)	0	0	1.6	0	0.8	5.6
Polymely (extra limb)	0	17.4	14.8	15.6	15.5	3.5
Femoral projection	0	0	3.3	4.4	3.1	0.7
Cutaneous fusion (skin webbing)	0	34.8	18.0	28.9	24.8	3.5
Taumely (bony triangle)	0	8.7	14.8	17.8	14.7	4.2
Micromely (smaller limb)	0	8.7	1.6	0	2.3	2.8
Limb hyperextension	0	0	1.6	2.2	1.6	0
Other malformations[a]	0	8.7	8.2	11.1	9.3	8.3
No. of abnormalities per abnormal toad[b]	0	1.77±0.23	2.35±0.25	2.5±0.28	2.26±0.16	1.12±0.04

Note: The value shown for each type of abnormality is the percentage of the total number of abnormalities observed. Experimental treatments are based on the number of parasites to which the animals were exposed: control (0 cercariae), light treatment (16 cercariae), intermediate treatment (32 cercariae), and heavy treatment (48 cercariae). The "combined" column presents the combined data from the three parasite treatments, while the "field" column presents data on abnormalities for toads from seven sites in the western U.S.A. Numbers in the parentheses indicate the number of abnormal toads / the number of toads inspected. The total number of abnormalities may or may not equal the number of abnormal animals, as many specimens had more than one abnormality.

[a]Includes clinodactyly (curved digit), syndactyly (fusion of digits), and anteversion (rotation of limb).

[b]Mean ± SE.

$\chi^2 = 14.87$, df = 3, $P < 0.0001$; Fig. 1). All control animals developed normally and suffered significantly less mortality than larvae in the combined *R. ondatrae* treatments (*G* test, $\chi^2 = 12.23$, df = 1, $P < 0.001$).

All malformations induced during the experiment affected the limbs, either fore, hind, or both. The diversity of limb abnormalities was remarkable, ranging from multiple supernumerary limbs to the complete absence of one or more limbs (Table 1, Fig. 2). In an extreme case, a toad exposed to an intermediate infection failed to develop three of its limbs and the one existing hind limb was duplicated distal to the femur. Cutaneous fusions (skin webbings) were the predominant malformation in all treatments (Fig. 2C, Table 1). Several individuals (5.3%) suffered cutaneous fusion of both hind limbs (*n* = 85). Polymely and taumely ranked as the second and third most common malformations, respectively (Figs. 2B and 2D, Table 1). The maximum number of extra limbs was two, and attachment of supernumerary hind limbs to the pelvis was equally distributed among the dorsal (35%),

ventral (30%), and medial (35%) surfaces (*n* = 20). Forelimb malformations, including extra and missing forelimbs, accounted for nearly 8% of observed abnormalities (Figs. 2E and 2F, Table 1).

Hind-limb polydactyly, another common malformation, usually occurred with taumely (80%; *n* = 20). As many as 12 digits were observed on a single foot. Toads with polydactyly or polypody invariably exhibited partial or complete mirror-image digit duplications in the anterior–posterior axis (Sessions et al. 1999; Johnson et al. 2001). Posterior mirror-image duplications (PMIDs) of the digit pattern 5-4-3-2-1-2-3-4-5 were more common than anterior mirror-image duplications (AMIDs) of the pattern 1-2-3-4-5-4-3-2-1 (52.4 and 38.1%, respectively; *n* = 21). Mirror-image triplications (MITs) of the pattern 1-2-3-4-5-4-3-2-1-2-3-4-5 accounted for the remaining 9.5% of digit duplications.

The severity-of-abnormality index, i.e., the mean number of abnormalities per abnormal toad, increased directly with *R. ondatrae* exposure (Table 1). The maximum number of

Fig. 1. Survivorship and malformation response of western toads (*Bufo boreas*) to infection with *Ribeiroia ondatrae*. Treatments were as follows: control (0 cercariae); light treatment (16 cercariae); intermediate treatment (32 cercariae); and heavy treatment (48 cercariae). Survivorship (▲, solid line) is calculated as the number of larvae surviving to metamorphosis divided by the initial sample size. The frequency of abnormalities (■, broken line) is calculated as the number of abnormal metamorphosing toads divided by the total number of metamorphosing toads within a treatment. Initial sample sizes were as follows: control, 60 larvae; light and intermediate treatments, 55 larvae each; heavy treatment, 50 larvae.
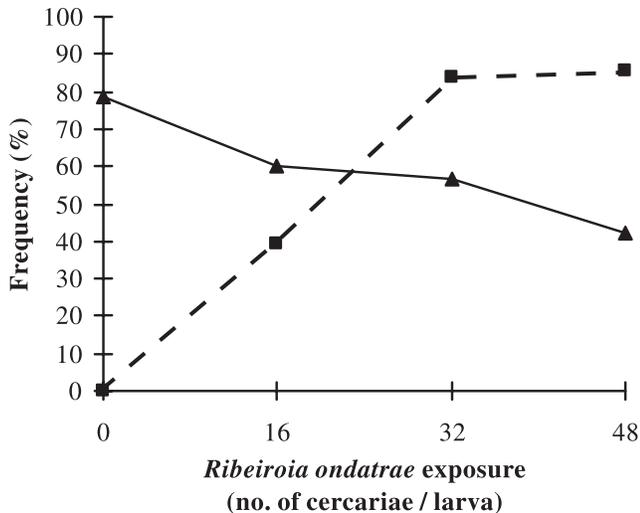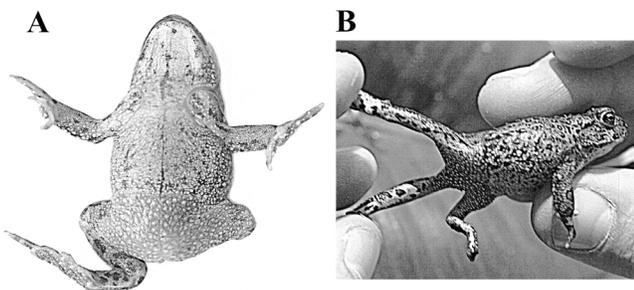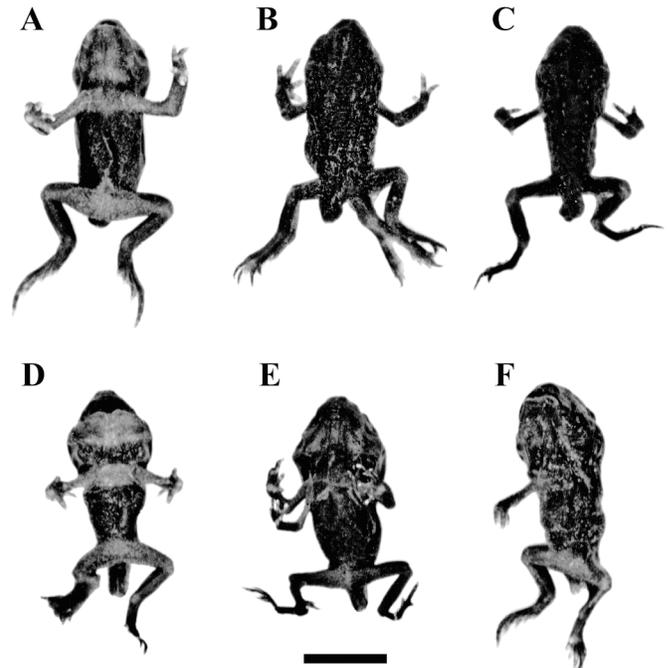

Fig. 2. Representative malformations produced in western toads exposed to varying numbers of *R. ondatrae* cercariae. B and C display the dorsal side of a metamorphic toad, while all remaining images offer a ventral view. (A) Control toad with normal limb development. (B) Toad with extra hind limbs (polymely) on the right side. (C) Toad with skin webbing (cutaneous fusion) in the right hind limb. (D) Toad with taumely in the right hind limb. (E) Toad with an extra right forelimb (polymely). (F) Toad with a missing left forelimb (ectromely). Scale bar = 10 mm.

Fig. 3. Abnormalities in wild-caught western toads. (A) Metamorphic toad exhibiting hemimely (a partially missing limb) on the left side. (B) Side view of a toad with hind-limb polymely (extra limb).

abnormalities recorded for an individual toad was five. Malformations were typically unilateral (66.7%), but the proportion of bilateral malformations increased with parasite dose. Rarely were malformations bilaterally symmetrical (3.5%; $n = 57$).

Between 1996 and 1999, abnormalities in larval and metamorphic western toads were also surveyed at three sites in California, three sites in Oregon, and one site in Washington as part of other studies (Johnson et al. 2001; Johnson et al.[2]). The frequency of abnormal toads varied by year and by pond, ranging from 2.8 to 11.1% (mean ± SE = 6.38 ± 1.07%; $n = 3189$). Each site supported *R. ondatrae*. To evaluate the role of *R. ondatrae* infection in causing these abnormalities, we compared abnormality types and their relative frequencies between combined field sites and our experi-

mental results using a percent similarity (PS) index (Jongman et al. 1995). Malformations induced in this experiment explained 30% of the relative composition of abnormalities from field sites ($PS_{ij} = 30.5$; Table 1). Within field sites, hind-limb ectrodactyly, hemimely (Fig. 3A), and apody constituted approximately 50% of the observed abnormalities. In contrast, the same categories accounted for little more than 3% of abnormalities in the experiment.

Unlike the other six sites, one pond exhibited a pattern that was closely aligned with the effects of *R. ondatrae* infection observed experimentally. The Washington site had an above-average malformation frequency (8.0%), with toads suffering from cutaneous fusion, polymely (Fig. 3B), taumely, and polydactyly, the four predominant malformations observed in the experiment. The PS_{ij} value between malformations observed at this site and those in the experiment was 72.4%. This site also supported an intensity of *R. ondatrae* infection of more than 50 metacercariae per toad, which exceeded the heaviest experimental exposure (48 cercariae) and was more than six times the mean intensity at any other site.

Discussion

The results of the current study corroborate those of a previous experiment with *R. ondatrae* and Pacific treefrogs, while also demonstrating the species-specific responses of western toads to trematode infection. In western toads, as in Pacific treefrogs, exposures to *R. ondatrae* cercariae caused

high frequencies of mortality and limb malformations. The frequency of malformations within a treatment was dose-dependent, increasing significantly with higher parasite exposures. Survivorship exhibited an inverse relationship with *R. ondatrae* infection, and only 42% of toads in the heavy treatment survived to metamorphosis. Considering (*i*) the similar response of both these anurans to *R. ondatrae* infection, (*ii*) the phylogenetic separation between the families Bufonidae and Hylidae (Duellman and Trueb 1986), and (*iii*) the field correlation between the presence of *R. ondatrae* and limb malformations in western amphibian species (Kaiser 1999; Johnson et al.[2]), we suspect that the parasite's teratogenic effects may be generalizable to many North American anurans.

Responses of anurans to *R. ondatrae* infection: western toads versus Pacific treefrogs

In spite of generally similar trends, the exact types of malformations and their relative frequencies differed between western toads and Pacific treefrogs. In response to identical levels of exposure, western toads exhibited several differences in the malformation pattern relative to that observed in Pacific treefrogs. While treefrog malformations were dominated by hind-limb polymely (45.2%) and hemi- and ectromely (19.4%) (Johnson et al. 1999), cutaneous fusion predominated among toad abnormalities, followed by hind-limb polymely and taumely (Table 1). Malformations induced in western toad metamorphs also included several types not observed in the Pacific treefrog experiment. Most intriguing of these were extra and missing forelimbs (Figs. 2E and 2F), as no forelimb anomalies were produced in infected treefrogs. Moreover, when malformations affected the hind limbs, experimental toads exhibited several other differences from treefrogs. Extra hind limbs were fewer (a maximum of two per toad) and emerged in nearly equal proportions from the dorsal, ventral, and medial surfaces of the pelvis. In field and experimental studies, Johnson et al. (1999, 2001) reported treefrogs with as many as eight hind limbs, and 95% of all extra limbs were attached dorsally to the pelvis (*n* = 764). Among toads with polydactyly or polypody, AMIDs and MITs, while less common than PMIDs, showed greater relative abundance than in Pacific treefrogs (Johnson et al. 2001).

Collectively, these differences in malformation types and their relative frequencies between Pacific treefrogs and western toads indicate that amphibian species may respond differently to identical numbers of *R. ondatrae* cercariae. The mechanism behind such differing responses is not yet known, but particular amphibian families or genera may have a greater propensity for certain types of malformations. Although mechanisms of limb development are largely conserved among vertebrates (Christen and Slack 1998), there may be significant differences in stage of initiation, relative size, and growth rate in amphibian limbs, even between those of species in the same genus (Blouin 1991). For example, extra forelimbs, which were produced in the current study with toads but not in a previous experiment with treefrogs, are virtually absent from historic reports of malformed hylids (but see O'Donoghue 1910). Nor have we observed any extra forelimbs among more than 1000 extra-legged Pacific treefrogs examined in the field (*n* = 17,353; Johnson et al. 2001; Johnson et al.[2]).

They are, however, relatively common among historic accounts involving malformed bufonids (Table 2).

Western toad larvae also demonstrated a weaker malformation response to *R. ondatrae* infection relative to the response of Pacific treefrogs (*G* test, $\chi^2 = 9.33$, df = 1, $P < 0.002$). Ninety-five percent of treefrogs in the intermediate exposure and 100% of those in the heavy treatment were malformed (Johnson et al. 1999), whereas only 83% of toad larvae exposed to intermediate parasite infections and 85% of those in the heavy treatment developed malformations. On the other hand, no significant differences were observed in survivorship between the two species following exposure to *R. ondatrae* (*G* test, $\chi^2 = 0$, df = 1, $P > 0.99$). The difference in sensitivity may be due to several behavioral or physical distinctions between the two anurans. Western toad larvae tend to be more active than those of Pacific treefrogs (Peterson and Blaustein 1992). During experimental exposures, toad larvae swam vigorously for much of the 120-min period, potentially reducing the ability of *R. ondatrae* cercariae to attach and penetrate the skin. In contrast, treefrog larvae were more sedate, swimming only sporadically. Physical differences among larvae, such as in size, epithelial properties, or developmental response to invading metacercariae, might be additionally or alternatively involved.

Data from studies of abnormalities in natural amphibian populations further suggest that western toads may be less affected by *R. ondatrae* than Pacific treefrogs. At ponds where Pacific treefrogs and western toads co-occurred, Pacific treefrogs exhibited higher intensities of *R. ondatrae* infection, higher frequencies of malformations, and more severe malformations than western toads (Johnson et al. 2001; Johnson et al.[2]). This disparity reflects either a reduced opportunity for *R. ondatrae* to utilize western toads as effective intermediate hosts or a reduced ability to do so. Pacific treefrogs, which have a long history of limb malformations in the field (Hebard and Brunson 1963; Miller 1968; Anderson 1977; Reynolds and Stephens 1984; Sessions and Ruth 1990; Johnson et al. 1999, 2001; Johnson et al.[2]), may be a more favored host of *R. ondatrae* for several reasons. First, toads are explosive breeders with a very short breeding season (Arnold and Wassersug 1978; Olson et al. 1997), which narrows the window of time during which *R. ondatrae* may infect larvae. Treefrogs are continuous breeders, with one of the longest breeding seasons of any western anuran (Duellman and Trueb 1986; Olson et al. 1997), greatly increasing the likelihood that cercariae will find appropriate stages of larvae to infect. An extended breeding season also allows more time for *R. ondatrae* infections in snails to achieve patency and for cercariae to be released. Toads and treefrogs also differ in skin properties. Toad epithelium contains noxious bufodienolides and is therefore unpalatable to many would-be predators (Duellman and Trueb 1986; Brodie and Formanowicz 1987; Lawler 1989; Mitchell et al. 1995; Alford 1999). These toxins are particularly effective against birds, fishes, and mammals, with mixed results for amphibian and invertebrate (e.g., insect larvae, leeches, crustaceans) predators (Voris and Bacon 1966; Arnold and Wassersug 1978; Brodie et al. 1978; Kruse and Stone 1984; Kats et al. 1988). An amphibian host that is unpalatable to birds and mammals could reduce or halt *R. ondatrae* transmission between its second intermediate (amphibian or fish) and

Table 2. Recent and historical reports of limb abnormalities in Bufonidae.

	Abnormality	Life-history stage	Frequency	Location	Reference
Bufo americanus	Missing limbs and digits	Metamorph	16.7% (*n* = 252)	Quebec, Canada	Ouellet et al. 1997
	Missing and malformed limbs	Metamorph	≈3%	Minnesota, U.S.A.	Hoppe and Mottl 1997
Bufo boreas	Missing limbs and digits	Adult	50% ("many")	California, U.S.A.	Storer 1925
	Extra hind limb	Adult	1	California, U.S.A.	Crosswhite and Wyman 1920
	Extra forelimb		1	Oregon, U.S.A.	Washburn 1899
	Extra hind limb	Metamorph	1		Bland-Sutton 1890
Bufo bufo	Symmetrical polydactyly	Adult	6.4% (*n* = 407)	Russia	Borkin and Pikulik 1986
	Extra forelimb	Adult	1	Russia	Borkin and Pikulik 1986
	Extra forelimb		1	Germany	Günther and Geiger 1996
	Polydactyly				Dubois 1979
	Extra forelimb	Adult	1	Germany	Meyer-Rochow and Koebke 1986
	Missing and malformed limbs and digits	Larva, metamorph	17.3% (*n* = 1 311)	Germany	Viertel and Veith 1992; Veith and Viertel 1993
	Missing and malformed limbs and digits	Larva, metamorph		Germany	Bohl 1997*a*
	Extra forelimb	Larva	0.006% (*n* = 15 000)	Germany	E. Bohl, personal communication
	Partially and completely missing hind limbs	Adult	0.3% (*n* = 10 000)	The Netherlands	Van Gelder and Strijbosch 1995
	Partially and completely missing limbs	Metamorph, adult	2.4% (*n* = 9 407)	United Kingdom	Wisniewski 1979
Bufo calamita	Missing limbs and digits		"rare"	Germany	Günther and Meyer 1996
Bufo gargarizans	Unilateral polydactyly		0.12% (*n* = 4 920)	Russia	Borkin and Pikulik 1986
Bufo marinus	Extra forelimb	Adult	2	Puerto Rico	Heatwole and Suarez-Lazu 1965
	Extra hind limb	Adult	1	Puerto Rico	Heatwole and Suarez-Lazu 1965
	Missing hind limb	Adult	1	Puerto Rico	Heatwole and Suarez-Lazu 1965
Bufo regularis	Extra hind limb	Adult	1	Egypt	Al-Hussaini 1953
Bufo viridis	Extra forelimb	Adult	1	Russia	Borkin and Pikulik 1986
	Extra limbs, missing and malformed limbs	Metamorph	50% (*n* = 100+)	Germany	Henle 1981

definitive hosts (birds and mammals), thereby selectively favoring infection of an alternative amphibian host species. The unpalatability of toads may be indirectly demonstrated by the large number of published reports documenting malformed adult toads (Table 2). Generally, abnormalities are suspected to impair the affected individual's ability to escape predation. Malformed adults are rare in other amphibian groups, presumably because they die before reaching maturity (see reviews by Taruffi 1885; Bateson 1894; Gemmill 1906; Van Valen 1974; Ouellet et al. 1997).

Relevance of experimental results to malformations in natural populations of toads

For well over a century, toads with abnormal limbs have been reported from around the world (Table 2). Reports concern nine species from six countries and encompass toads with extra, missing, and malformed fore and hind limbs. Two reports document an extra limb in western toads. Washburn (1899) described a western toad from Oregon with an extra right forelimb and Crosswhite and Wyman (1920) presented a sketch of a California specimen with an extra hind limb. Both malformations are similar to those produced in this experiment and may have resulted from *R. ondatrae* infection. Unfortunately, the whereabouts of these specimens, and even whether they were preserved, are unknown. Other accounts, however, particularly those documenting numerous toads with symmetrical polydactyly, are distinct from experimental effects of *R. ondatrae* (Table 2; Dubois 1979; Borkin and Pikulik 1986). Rostand (1958) linked the influence of various alleles to several such occurrences and, in studies with *Rana esculenta*, determined that extracts from fish (*Tinca* sp., *Anguilla* sp.) intestines induced a similar yet more severe phenomenon in exposed larvae ("anomalie P" in Rostand 1971; Dubois 1979). Laboratory experiments with toad larvae have isolated other agents capable of causing symmetrical hind-limb anomalies. Bruschelli and Rosi (1971) discovered that low doses of vitamin A could cause symmetrical duplication of the hind limbs, while Muto (1969) induced bilateral reductions of the digits, feet, and distal limb segments by exposing larvae to high temperatures (30°C).

At our own study sites in the western U.S.A., the role of *R. ondatrae* infection in causing the abnormalities observed in wild western toad populations may be case-dependent. Nearly 10% of western toad metamorphs at a pond in northern Washington State exhibited malformations similar to those produced in the present experiment: skin webbings, extra hind limbs, taumely, and extra digits. This site also supported a greater intensity of *R. ondatrae* infection than other ponds we surveyed. At the remaining six sites, however, malformations similar to those induced by *R. ondatrae* infection accounted for only 30% of recorded limb abnormalities. Hemimely, the most common abnormality among combined field sites, was not produced in this experiment (Fig. 3A, Table 1). This suggests that, while *R. ondatrae* infection may cause a percentage of the abnormalities at these sites, the agent(s) causing the majority are not yet known.

Attempted predation by small fishes, leeches, and aquatic insects is likely to account for some of the abnormalities. Although larvae attacked by predators are frequently consumed, they occasionally escape with only damaged limbs. Certain crustaceans and other invertebrate predators attack anuran limbs, sometimes causing limb loss or injury (Licht 1974; Formanowicz and Brodie 1982; Duellman and Trueb 1986). Unlike salamanders, anurans exhibit diminished regenerative abilities as larvae approach metamorphosis (Michael and El Mekkawy 1977; Muneoka et al. 1986), and limb injury followed by partial regeneration may result in severe malformations. Depending on the stage of the larva, incomplete regeneration of the damaged limb(s) can lead to cutaneous fusion, anteversion, or micromely, even in the absence of other teratogens (Bohl 1997a; Tyler 1998).

Recently, independent researchers in several parts of Germany identified a leech (*Erpobdella octoculata*) as the cause of high frequencies of limb abnormalities in the common toad *Bufo bufo* (Table 2; Viertel and Veith 1992; Veith and Viertel 1993; Bohl 1997a, 1997b). In all studies, partially and completely missing limbs accounted for more than 50% of the abnormalities. In these studies, extensive water-quality testing, breeding experiments, electrophoretic analyses, reciprocal translocations of larvae between affected and control sites, predator exclosures, and laboratory predation trials were collectively performed (Viertel and Veith 1992; Veith and Viertel 1993; Bohl 1997a). While no genetic or chemical effects were observed, predator exclosures prevented the occurrence of abnormalities in caged animals (Bohl 1997a). In laboratory predation trials with *E. octoculata* and *B. bufo* larvae, the types of abnormalities observed in wild toads were reproduced (Viertel and Veith 1992). Even more convincing, a substantial reduction of the pond's leech population led to the development of completely normal *B. bufo* larvae and metamorphs the following spring (Bohl 1997b). In reviewing reports of toad abnormalities from other parts of Germany, Veith and Viertel (1993) concluded that leech attacks were the likely cause of cases described in Flindt (1985), Grosse and Bauch (1988), and Anonymous (1991). Van Gelder and Strijbosch (1995) arrived at a similar conclusion for a site with abnormal toads in the Netherlands.

Nor are leeches the only predators capable of causing such abnormalities in toads. As part of another study, one of us (J.B.) recorded high levels (1–35%; *n* = 7987) of limb abnormalities in a western toad population in central Oregon between 1988 and 1999. Although *R. ondatrae* were recorded from toad larvae in this pond, infection intensities were extremely low (mean ± SE = 0.33 ± 0.3). Abnormalities consisted primarily of missing limbs and digits. Instead of leeches however, the implicated predators were fish (sticklebacks (*Gasterosteus aculeatus*)), which achieved high densities following their introduction in the early 1980s. Preliminary experiments with predator exclosures prevented limb abnormalities in caged larvae, and laboratory predation experiments demonstrated that sticklebacks will attack and injure the developing hind limbs of western toads. Abnormalities resulting from these encounters were similar to those observed in the field. Correspondingly, years of low stickleback density preceded seasons with low frequencies of abnormalities in emerging toads.

At our other field sites, the toad-limb abnormalities unexplained by *R. ondatrae* infection are similar to those described in the leech and stickleback studies discussed above. In all cases, abnormalities primarily affect the hind limbs, are typically unilateral, and are dominated by missing and partially missing limbs and digits. While testing negative for

pesticides, polychlorinated biphenyls (PCBs), and heavy metals (Johnson et al. 1999; Johnson et al.[2]), each site supports a number of candidate predators. Three ponds support dense populations of mosquitofish (*Gambusia affinis*), which attack Pacific treefrog and California newt (*Taricha torosa*) larvae in laboratory and field experiments (Gamradt and Kats 1996; Goodsell and Kats 1999). *Erpobdella punctata*, the North American congener of *E. octoculata*, has also been identified from several of our sites. Experiments involving controlled predation trials in the laboratory and exclosure studies in the field are needed to determine what role, if any, these and other anuran predators have on limb development in western toads.

In conclusion, our results describe the effects of *R. ondatrae* infection on a second anuran species. Western toad larvae exposed to *R. ondatrae* cercariae exhibited low survivorship and a high frequency of malformations. The resulting pattern of abnormalities differed from that induced in Pacific treefrogs both in the types of malformations produced and in their relative frequencies (Johnson et al. 1999). The effects of *R. ondatrae* infection on other anuran families have not been tested, but the current findings, coupled with the results of recent field studies on *R. ondatrae* infection and amphibian malformations (Kaiser 1999; Johnson et al.[2]), suggest that individual species may respond differently to a single agent. In future experiments, the specific effects of *R. ondatrae* on various ranids need to be examined, as species of this group have been frequently reported with limb malformations in the midwestern U.S.A. and Canada (Hoppe and Mottl 1997; Ouellet et al. 1997; Burkhart et al. 1998; Helgen et al. 1998). The role of trematode infection in these reports is not clear, but Sutherland (2001; unpublished data) recently isolated *R. ondatrae* metacercariae in malformed mink frogs (*Rana septentrionalis*) and northern leopard frogs (*Rana pipiens*) from two Minnesota sites with a history of amphibian malformations. These findings highlight the need for further studies on the connection between *R. ondatrae* infection and amphibian limb malformations in North America. Although *R. ondatrae* is not an invasive trematode in North America, the possibility of a recent increase in the geographic prevalence of the parasite or its infection intensity in amphibians deserves consideration. Many consequences of human activity, including cultural eutrophication, alteration of aquatic systems or construction of artificial systems, and introduction of snails, can dramatically alter the distribution and severity of trematode-related maladies (Johnson and Lunde 2001).

References

Alford, R.A. 1999. Ecology: resource use, competition, and predation. *In* Tadpoles: the biology of anuran larvae. *Edited by* R.W. McDiarmid and R. Altig. The University of Chicago Press, Chicago. pp. 241–278.

Al-Hussani, A.H. 1953. A case of polymely in the Egyptian toad, *Bufo regularis*. Bull. Zool. Soc. (Egypt), **11**: 48–51.

Anderson, M.E. 1977. Aspects of the ecology of two sympatric species of *Thamnophis* and heavy metal accumulation within the species. M.S. thesis, University of Montana, Missoula.

Ankley, G.T., Tietge, J.E., DeFoe, D.L., Jensen, K.M., Holcombe, G.W., Durhan, E.J., and Diamond, S.A. 1998. Effects of ultraviolet light and methoprene on survival and development of *Rana pipiens*. Environ. Toxicol. Chem. **17**: 2530–2542.

Anonymous. 1991. Amphibien am Scheideweg. Natur und Umwelt, Bonn, **71**: B4-B5.

Arnold, S.J., and Wassersug, R.J. 1978. Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): social behavior as a possible defense. Ecology, **59**: 1014–1022.

Basch, P.F., and Sturrock, R.F. 1969. Life history of *Ribeiroia marini* (Faust and Hoffman, 1934) comb.n. (Trematoda: Cathaemasiidae) J. Parasitol. **55**: 1180–1184.

Bateson, W. 1894. Materials for the study of variation. MacMillan and Co., New York.

Beaver, P.C. 1939. The morphology and life history of *Psilostomum ondatrae* Price 1931 (Trematoda: Psilostomatidae). J. Parasitol. **25**: 383–393.

Bland-Sutton, J. 1890. Evolution and disease. Walter Scott, London.

Blaustein, A.R., Hokit, D.G., O'Hara, R.K., and Holt, R.A. 1994. Pathogenic fungus contributes to amphibian losses in the Pacific Northwest. Biol. Conserv. **67**: 251–254.

Blouin, M.S. 1991. Proximate developmental causes of limb length variation between *Hyla cinerea* and *Hyla gratiosa* (Anura: Hylidae). J. Morphol. **209**: 305–310.

Bohl, E. 1997*a*. Limb deformities of amphibian larvae in Aufseß (Upper Franconia): attempt to determine causes. *In* Munich contributions to wastewater fishery and river biology. R. Oldenbourg Verlag GmbH, Munich. pp. 160–189.

Bohl, E. 1997*b*. Erfolgskontrolle der Egeldichtereduktion im Naturteich des Fischereibeispielbetriebs in Aufseß (Oberfranken, Landkreis Bayreuth). Bericht von Diplom Biologe Roland Fischer, Bayreuth, Germany (September).

Borkin, L.J., and Pikulik, M.M. 1986. The occurrence of polymely and polydactyly in natural populations of anurans in the U.S.S.R. Amphib.-Reptilia, **7**: 205–216.

Brodie, E.D., Jr., and Formanowicz, D.R., Jr. 1987. Antipredator mechanisms of larval anurans: protection of palatable individuals. Herpetologica, **43**: 369–373.

Brodie, E.D., Jr., Formanowicz, D.R., Jr., and Brodie, E.D., III. 1978. The development of noxiousness of *Bufo americanus* tadpoles to aquatic insect predators. Herpetologica, **34**: 302–306.

Bruschelli, G.M., and Rosi, G. 1971. Polymelia induced by vitamin A in embryos of *Bufo vulgaris*. Rivista di Biologia, **64**: 271–283.

Burkhart, J.G., Helgen, J.C., Fort, D.J., Gallagher, K., Bowers, D., Propst, T.L., Gernes, M., Magner, J., Shelby, M.D., and Lucier, G. 1998. Induction of mortality and malformation in *Xenopus laevis* embryos by water sources associated with field frog deformities. Environ. Health Perspect. **106**: 841–848.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Vol. 1. 2nd ed. Canadian Council on Animal Care, Ottawa, Ont.

Carey, C. 1993. Hypothesis concerning the causes of the disappear-

ance of boreal toads from the mountains of Colorado. Conserv. Biol. **7**: 355–362.

Christen, B., and Slack, J.M.W. 1998. All limbs are not the same. Nature (Lond.), **395**: 230–231.

Crosswhite, E., and Wyman, M. 1920. Notes on an abnormal toad. J. Entomol. Zool. Pomona College, **12**: 78.

Drost, C.A., and Fellers, G.M. 1996. Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada, U.S.A. Conserv. Biol. **10**: 414–425.

Dubois, A. 1979. Anomalies and mutations in natural populations of the *Rana* "*esculenta*" complex (Amphibia, Anura). Mitt. Zool. Mus. Berl. **55**: 59–87.

Duellman, W.E., and Trueb, L. 1986. Biology of amphibians. The John Hopkins University Press, Baltimore, Md.

Flindt, R. 1985. Untersuchungen zum Auftreten von mißgebildeten Wechselkroten (*Bufo viridis*) in einem Steinbruch in Vahingen-Roßwag. Jahrb. Ges. Naturkd. Wurttemb. Stuttg. **140**: 211–233.

Formanowicz, D.R., Jr., and Brodie, E.D., Jr. 1982. Relative palatabilities of members of a larval amphibian community. Copeia, 1982: 91–97.

Fort, D.J., Propst, T.L., Stover, E.L., Helgen, J.C., Levey, R.B., Gallagher, K., and Burkhart, J.G. 1999. Effects of pond water, sediment, and sediment extracts from Minnesota and Vermont, U.S.A., on early development and metamorphosis of *Xenopus*. Environ. Toxicol. Chem. **18**: 2305–2315.

Gamradt, S.C., and Kats, L.B. 1996. Effect of introduced crayfish and mosquitofish on California newts. Conserv. Biol. **10**: 1155–1162.

Gardiner, D.M., and Hoppe, D.M. 1999. Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). J. Exp. Zool. **284**: 207–216.

Gemmill, J.F. 1906. Supernumerary limb in a frog. J. Anat. Physiol. **40**: 387–395.

Goodsell, J.A., and Kats, L.B. 1999. Effect of introduced mosquitofish on Pacific treefrogs (*Hyla regilla*) and the role of alternative prey. Conserv. Biol. **13**: 921–924.

Gosner, K.L. 1960. A simplified table for staging anuran larvae with notes on identification. Herpetologica, **16**: 183–190.

Grosse, W.R., and Bauch, I. 1988. Fehlentwicklung beim Laubfrosch *Hyla arborea*. L. Feldherpetol. (Erfurt), 1988: 25–28.

Günther, R., and Geiger, A. 1996. Artkapitel Amphibien Erdkröte : *Bufo bufo*. *In* Die Amphibien und Reptilien Deutschlands. *Edited by* R. Günther. Gustav Fisher Verlag, Jena, Germany. pp. 274–301.

Günther, R., and Meyer, F. 1996. Artkapitel Amphibien Kreuzkröte : *Bufo calamita*. *In* Die Amphibien und Reptilien Deutschlands. *Edited by* R. Günther. Gustav Fisher Verlag, Jena, Germany. pp. 302–321.

Heatwole, H., and Suarez-Lazu, P.J. 1965. Supernumerary legs in *Bufo marinus* and abnormal regeneration of the tail in *Ameiva exsul*. J. Ohio Herpetol. Soc. **5**: 30–31.

Hebard, W.B., and Brunson, R.B. 1963. Hind limb anomalies of a western Montana population of the Pacific treefrog, *Hyla regilla*. Copeia, 1963: 570.

Helgen, J.C., McKinnell, R.G., and Gernes, M.C. 1998. Investigation of malformed northern leopard frogs in Minnesota. *In* Status and conservation of midwestern amphibians. *Edited by* M.J. Lannoo. University of Iowa Press, Iowa City. pp. 288–297.

Henle, K. 1981. A unique case of malformations in a natural population of the green toad (*Bufo viridis*) and its meaning for environmental politics. Br. Herpetol. Soc. Bull. **4**: 48–49.

Hoppe, D.M. 2000. History of Minnesota frog abnormalities: do recent findings represent a new phenomenon? J. Iowa Acad. Sci. **107**: 86–89.

Hoppe, D.M., and Mottl, E. 1997. Anuran species differences in malformation frequency and severity in Minnesota. *In* Abstracts from the 18th Annual Meeting of the Society of Toxicology and Chemistry, San Francisco. p. 90. [Abstr.]

Johnson, P.T.J., and Lunde, K.B. 2001. Trematode parasites and amphibian limb malformations in the western U.S.: are they a concern? *In* Status and conservation of U.S. amphibians. *Edited by* M.J. Lannoo. University of California Press, Berkeley. In press.

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 (Washington, D.C.), **284**: 802–804.

Johnson, P.T.J., Lunde, K.B., Ritchie, E.G., Reaser, J.K., and Launer, A.E. 2001. Morphological abnormality patterns in a California amphibian community. Herpetologica. In press.

Jongman, R.H.G., Ter Braak, C.J.F., and Van Tongeren, O.F.R. 1995. Data analysis in community and landscape ecology. Cambridge University Press, Cambridge and New York.

Kaiser, J. 1997. Deformed frogs leap into spotlight at health workshop. Science (Washington, D.C.), **278**: 2051–2052.

Kaiser, J. 1999. Link to parasites grows stronger. Science (Washington, D.C.), **286**: 2434.

Kats, L.B., Petranka, J.W., and Sih, A. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. Ecology, **69**: 1865–1870.

Kruse, K.C., and Stone, B.M. 1984. Largemouth bass (*Micropterus salmoides*) learn to avoid feeding on toad (*Bufo*) tadpoles. Anim. Behav. **32**: 1035–1039.

Lawler, S.P. 1989. Behavioral responses to predator and predation risk in four species of larval anurans. Anim. Behav. **38**: 1039–1047.

Licht, L.E. 1974. Survival of embryos, tadpoles, and adults of the frogs *Rana aurora aurora* and *Rana pretiosa pretiosa* sympatric in southwestern British Columbia. Can. J. Zool. **52**: 613–627.

Martof, B. 1956. Factors influencing size and composition of populations of *Rana clamitans*. Am. Midl. Nat. **56**: 224–245.

Meyer-Rochow, V.B., and Asashima, M. 1988. Naturally occurring morphological abnormalities in wild populations of the Japanese newt *Cynops pyrrhogaster* (Salamandridae: Urodela: Amphibia). Zool. Anz. **221**: 70–80.

Meyer-Rochow, V.B., and Koebke, J. 1986. A study of the extra extremity in a five-legged *Rana temporaria* frog. Zool. Anz. **217**: 1–13.

Michael, M.I., and El Mekkawy, D.A. 1977. Progress of malformations during limb regeneration in stages of the Egyptian toad, *Bufo regularis* Reuss, after transection at various proximo-distal levels. Mem. Soc. Zool. Fr. **41**: 203–208.

Miller, C.E. 1968. Frogs with five legs. Carol. Tips, **31**: 1.

Mitchell, D., Jones, A., and Hero, J.M. 1995. Predation on the cane toad (*Bufo marinus*) by the black kite (*Milvus migrans*). Mem. Queensl. Mus. **38**: 512.

Muneoka, K., Holler-Dinsmore, G., and Bryant, S.V. 1986. Intrinsic control of regenerative loss in *Xenopus laevis* limbs. J. Exp. Zool. **240**: 47–54.

Muto, Y. 1969. Anomalies in the hindlimb skeletons of the toad larvae reared at a high temperature. Congenital Anom. **9**: 61–72.

O'Donoghue, C. 1910. Instances of polymely of two frogs. Zool. Anz. **35**: 759–766.

Olson, D.H., Leonard, W.P., and Bury, R.B. 1997. Sampling amphibians in lentic habitats: methods and approaches for the Pacific Northwest. Society for Northwestern Vertebrate Biology, Olympia, Wash.

Ouellet, M., Bonin, J., Rodrigue, J., DesGranges, J., and Lair, S. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free

living anurans from agricultural habitats. J. Wildl. Dis. **33**: 95–104.

Peterson, J.A., and Blaustein, A.R. 1992. Relative palatabilities of anuran larvae to natural aquatic insect predators. Copeia, 1992: 577–584.

Reynolds, T.D., and Stephens, T.D. 1984. Multiple ectopic limbs in a wild population of *Hyla regilla*. Great Basin Nat. **44**: 166–169.

Rostand, J. 1958. Les anomalies des amphibiens anoures. Sedes, Paris.

Rostand, J. 1971. Les étangs Ömonstres : histoire d'une recherche (1947–1970). Stock, Paris.

Sessions, S.K., and Ruth, S.B. 1990. Explanation for naturally occurring supernumerary limbs in amphibians. J. Exp. Zool. **254**: 38–47.

Sessions, S.K., Franssen, R.A., and Horner, V.L. 1999. Morphological clues from multilegged frogs: are retinoids to blame? Science (Washington, D.C.), **284**: 800–802.

Stebbins, R.C., and Cohen, N.W. 1995. A natural history of amphibians. Princeton University Press, Princeton, N.J.

Storer, T.I. 1925. A synopsis of the Amphibia of California. University of California Press, Berkeley.

Sutherland, D. 2001. Parasites of North American anurans. *In* Status and conservation of U.S. amphibians. *Edited by* M.J. Lannoo. University of California Press, Berkeley. In press.

Taruffi, C. 1885. Storia della teratologia. Tomo I–IV. Regia Tipografía, Bologna, Italy.

Tietge, J.E., Ankley, G.T., Defoe, D.L., Holcombe, G.W., and Jensen, K.M. 2000. Effects of water quality on development of *Xenopus laevis*: a frog embryo teratogenesis assay—*Xenopus* assessment of surface water associated with malformations in native anurans. Environ. Toxicol. Chem. **19**: 2114–2121.

Tyler, M.J. 1998. Frogs as environmental monitoring organisms. *In* Australian frogs: a natural history. *Edited by* M.J. Tyler. Cornell University Press, Ithaca, N.Y. pp. 141–160.

Van Gelder, J.J., and Strijbosch, H. 1995. Adult common toads (*Bufo bufo*) with mutilated legs. Alytes, **13**: 105–108.

Van Valen, L. 1974. A natural model for the origin of some higher taxa. J. Herpetol. **8**: 109–121.

Veith, M., and Viertel, B. 1993. Veränderungen an den Extremitäten von Larven und Jungtieren der Erdkröte (*Bufo bufo*) : Analyse möglicher Ursachen. Salamandra, **29**: 184–199.

Viertel, B., and Veith, M. 1992. Predation by leeches and regeneration, a factor in larval development of *Bufo bufo* (L.). *In* Proceedings of the Sixth Ordinary General Meeting of the Societas Europaea Herpetologica, Budapest, 1991. *Edited by* Z. Korsos and I. Kiss. Hungarian Natural History Museum, Budapest. pp. 479–484.

Voris, H.K., and Bacon, J.P. 1966. Differential predation on tadpoles. Copeia, 1966: 594–598.

Washburn, F.L. 1899. A peculiar toad. Am. Nat. **33**: 139–141.

Wisniewski, P.J. 1979. Notes on leg-loss in the common toad. ASRA J. **1**: 43–47.