ECOTOXICOLOGY OF AMPHIBIANS AND REPTILES

Edited by

Donald W. Sparling
Greg Linder
Christine A. Bishop

A Technical Publication of

SETAC®

CHAPTER 11

Amphibian Deformities: Current State of Knowledge

Martin Ouellet

Isolated cases of amphibian deformities have been reported in scientific literature for nearly 300 years. Vallisneri (1733b, 1733c) recorded the occurrence of a frog with an additional limb, first described in 1706. In this particular frog, the fifth limb was located in the right pelvic region and the left hindlimb was polydactylous with seven toes (see Appendix). The specimen was caught in Scandiano, a little town located in the region of Emilia-Romagna in northern Italy. In 1740, De Superville mentioned a frog with a supernumerary limb at the level of the right shoulder. This case and two others (published in 1783 by Guettard, and 1816 by Otto) were cited by Geoffroy Saint-Hilaire (1836b) in the third volume of his classic treatise on monstrosities in humans and animals. Virey (1819) and Geoffroy Saint-Hilaire (1832) also cited instances of polydactyly in frogs and salamanders, respectively. Duméril (1865a) reviewed early observations of deformed anurans, most of them carrying supernumerary limb structures, and described in detail 10 cases that were known to him, although at least 17 extra-legged frogs had in fact been documented by 1865 (Taruffi 1880; Ercolani 1881). Taruffi (1880) presented a good historic review, tabulating 32 observations of polymelous anurans by year of publication from 1706 until 1879. In North America, the first described abnormal amphibian was a five-legged *Rana pipiens* thought to be found in Rochester, New York, around 1850 (Kingsley 1881).

More contemporary reviews of supernumerary limbs and other deformities in amphibians are those of Bateson (1894), Gemmill (1906), Przibram (1921), Woitkewitsch (1959), Van Valen (1974), Vizotto et al. (1977), and Dubois (1979). Past records of polymelous frogs found in Japan were recently presented by Takeishi (1996). A well-illustrated atlas of anuran deformities was produced by Rostand (1958). An atlas of developmental abnormalities also exists for *Xenopus laevis* embryos raised under laboratory conditions (Bantle et al. 1991). A general review of color abnormalities in free-living anurans such as albinism, melanism, black eyes, and blue frogs is given by Dubois (1979). Although most of these reports refer to individual cases of deformed amphibians, the recent literature concentrates more on the description of mass occurrences of deformities in particular populations. It is

Ecotoxicology of Amphibians and Reptiles. Donald W. Sparling et al., editors.
 ISBN 1-880611-28-7

impossible to know how many of the historic mentions were part of population level phenomena.

Morphological abnormalities and injuries occur normally at low frequencies in wild populations of amphibians, ranging from 0 to 2% (Rostand 1949b; Dubois and Vachard 1969; Koskela 1974; Dubois 1979; Semlitsch et al. 1981; Borkin and Pikulik 1986; Luis and Báez 1987; Meyer-Rochow and Asashima 1988; Read and Tyler 1994; Marvin and Hutchison 1997). For example, 451 cases (1.0%) of clinodactyly, ectrodactyly, and polydactyly were recorded in a sample of 44,000 adult *Bufo bufo* examined from two regions of France (Rostand 1949b). Meyer-Rochow and Asashima (1988) reported 2.4% or 335 cases of external deformities (ectrodactyly, polydactyly, polymely, abnormal webbing of toes, and tail projections) for a total of 13,815 adult *Cynops pyrrhogaster* newts from Japan. Of 4137 frogs examined from different age classes, eight cases (0.2%) of unilateral anophthalmia were found in populations of *Rana esculenta* complex (Dubois 1979). In his standard table for staging anuran embryos and larvae, Gosner (1960) mentioned that aberrant mouth parts were common in some samples (see also Grillitsch and Grillitsch 1989).

However, this is not the phenomenon of concern. It is only when frequencies of abnormalities grossly exceed the baseline level that there is any reason to be alarmed. Recently, an increasing number of populations where frequencies of deformities were abnormally high (> 5%) have been described in parts of North America, Europe, and Asia. Up to 85% of the individuals from a given population can show external abnormalities (Rostand 1959, 1971; Rostand and Darré 1968; Mizgireuv et al. 1984; Flindt 1985; Vershinin 1989, 1995b; Sessions and Ruth 1990; Veith and Viertel 1993; Bohl 1997; Flax and Borkin 1997; Ouellet et al. 1997; Burkhart et al. 1998; Helgen et al. 1998; Johnson et al. 1999). Many factors have been proposed or shown to cause developmental abnormalities in amphibians, but further studies will be necessary to explain the exact mechanisms and developmental pathways involved. The ecological significance of several of these factors also remains to be proven. Whatever the actual causes of such deformities, the biggest challenge that remains is to determine if their origins are natural or anthropogenic.

The apparent global decline of amphibians and the consequent growth of interest in monitoring amphibian populations have stimulated numerous studies around the world (Kuzmin et al. 1995; Green 1997; Lannoo 1998). Are amphibian deformities more common simply because of increased surveillance, or are they recent and widespread phenomena? Are deformities related to the worldwide decline of amphibian populations? Are natural causes responsible, or are we ourselves contributing to ecosystem degradation, with possible relevance to human health?

To better appreciate the difficulty and complexity of answering these questions, I have reviewed the existing literature on amphibian deformities up to early 1999. Almost exclusively, peer-reviewed and published material has been considered. I have standardized the technical terms used to describe these abnormalities (see

Appendix). The terms abnormalities, anomalies, malformations, malformities, and monstrosities were used interchangeably in the literature to refer to cases of amphibian deformities. I focused on all external deformities involving the eyes, head, limbs, mouth, oral cavity, snout, and tail. Accounts of color mutations, diseases, neoplasms, and internal abnormalities were not included in this review. I had multiple objectives. First, I reviewed the reports of deformed frogs, toads, newts, and salamanders in the wild to seek generalities of species sensitivity and geographic distribution. Second, the possible causes and hypotheses used to explain deformities were explored and analyzed. Third, I also examined the implications of developmental abnormalities with regard to amphibian population declines, ecosystem degradation, and human-health concerns. Finally, I conclude with recommendations and suggestions for future research on amphibian deformities.

Species Sensitivity and Geographic Distribution

A total of 67 different species of anurans and 26 species of salamanders with deformities have been documented (Tables 11-1 and 11-2). Many of the accounts are purely descriptive without any analysis. One hundred and forty-one reports out of a total of 202 (69.8%) refer to deformities in fewer than 10 individuals per species at particular sites (Figure 11-1), and many of these describe only a single case. At the other extreme, 17 situations (8.4%) have deformity occurrences at one or more sites greater than 100 individuals per species. Some reports may refer to the same

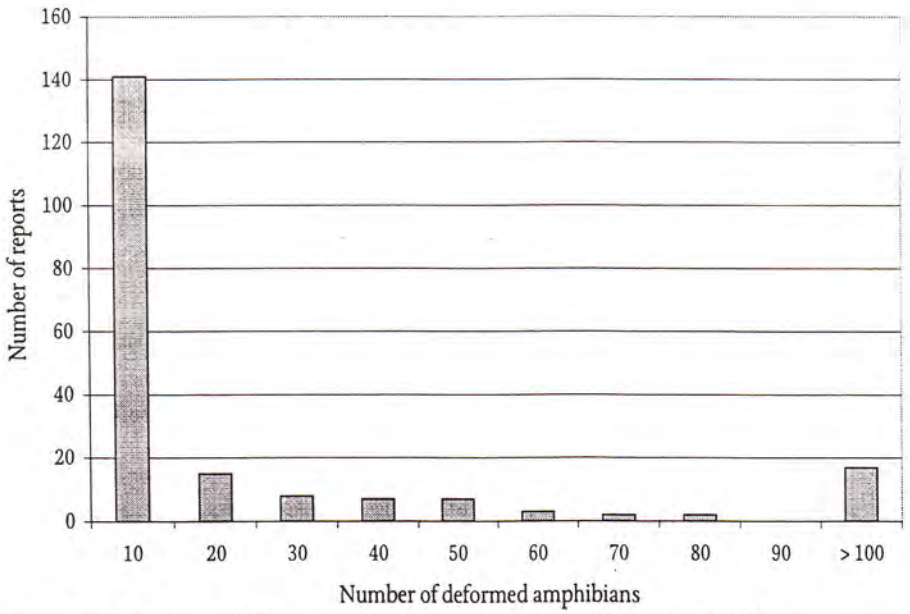

Figure 11-1 Numbers of published reports describing numbers of deformed amphibians

Table 11-1 Species sensitivity and geographic distribution of published frog deformities in the wild

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
Bufonidae					
Bufo americanus	E	1	A	Canada (ON)	Rollo 1995
	L^{E}	42	M	Canada (QC)	Ouellet et al. 1997
B. arenarum	L^{P}	1	A	Argentine Republic	Gaggero 1960
B. boreas	L^{P}	1	A	United States (OR)	Washburn 1899
	L^{P}	1	A	United States (CA)	Crosswhite and Wyman 1920
B. bufo	L^{P}	1	M	United Kingdom	Bland Sutton 1889, 1890
	$L^{E,P}$*, S	> 461	A, M	France	Rostand 1949b, 1951a, 1958, 1971
	L^{P}	> 6	A	France	Dubois 1974
	L^{P}	4	A	Byelorussia	Borkin and Pikulik 1986
	L^{E}*	226	M, T	Federal Republic of Germany	Veith and Viertel 1993
B. ictericus	L^{P}	1	A	Federative Republic of Brazil	Pasquarelli et al. 1981
B. maculatus	L^{E}	6	A, M	Zimbabwe	Lambiris 1982
B. marinus	$L^{E,P}$	4	A	Puerto Rico[4]	Heatwole and Suárez-Lazú 1965
	L^{E}	1	A	United States (HI)[4]	Chan and Young 1985
B. melanostictus	L^{P}	1	A	Sri Lanka	Samarasinghe 1951
B. regularis	L^{P}	1	A	Arab Republic of Egypt	Al-Hussaini 1953
	L^{P}	1	A	Arab Republic of Egypt	Ghorab 1959
B. viridis	$L^{E,P}$, M	> 50	M, T	Federal Republic of Germany	Henle 1981
	$L^{E,P}$	> 51	A, M	Federal Republic of Germany	Flindt 1985
	L^{P}	1	A	Russian Federation	Dunayev 1997
	L^{E}	2	A or M	Ukraine	Flax and Borkin 1997
Dendrobatidae					
Mannophryne lamarcai	L^{E}*	7	A	Venezuela	Mijares-Urrutia and Arends 1999

Table 11-1 continued

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
Discoglossidae					
Alytes obstetricans	L^{P}	1	A or M	Spain	Cisternas 1865
	E	1	T	France	Héron-Royer 1884
	L^{P}*	1	M	Federal Republic of Germany	Hellmich 1929
	L^{P}	1	A	France	Rostand 1955c, 1958
Bombina bombina	L^{E}	567	A or M	Ukraine	Flax and Borkin 1997
B. orientalis	L	1	A or M	Democratic People's Republic of Korea	Tyler 1989
Discoglossus pictus	L^{P}	1	A	Spain	Sanchiz and Pérez 1974
Hylidae					
Acris crepitans	E	1	A	United States (MO)	Smith and Powell 1983
Cyclorana australis	E, L^{E}*	68	M	Australia	Tyler 1989
C. longipes	L^{E}*	39	A, M	Australia	Tyler 1989
Hyla meridionalis	L^{E}*	5	A or M	Spain [Canary Islands]	Luis and Báez 1987
H. regilla	L^{P}	> 18	M	United States (MT)	Hebard and Brunson 1963
	L^{P}	> 5	M	United States (WA)	Miller 1968
	L^{P}	13	M	United States (ID)	Reynolds and Stephens 1984
	$L^{E, P}$*	205	A, M, T	United States (CA)	Sessions and Ruth 1990
	E, $L^{E, P}$, M	1086	M	United States (CA)	Johnson et al. 1999
	L^{P}	391	M	United States (CA, OR)	Sessions et al. 1999
Litoria aurea	L^{P}	1	A	Australia	O'Donoghue 1910
	L	1	A	New Zealand	Richardson and Barwick 1957
L. bicolor	L^{E}	6	A or M	Australia	Tyler 1989
L. caerulea	L^{E}, M	4	A or M	Australia	Tyler 1989
L. dahlii	L^{E}*	11	A or M	Australia	Tyler 1989
L. inermis	L^{E}	7	A or M	Australia	Tyler 1989
L. meiriana	L^{E}	3	A or M	Australia	Tyler 1989
L. pallida	L^{E}*	5	A or M	Australia	Tyler 1989
L. rothii	L^{E}	1	A or M	Australia	Tyler 1989

Table 11-1 continued

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
L. rubella	L^{P}	1	A	Australia	Tyler 1976
	L^{E}*, M	36	A or M	Australia	Tyler 1989
L. tornieri	L^{E}	4	A or M	Australia	Tyler 1989
Phrynohyas hebes	A, L^{P}	1	A	Federative Republic of Brazil	Vizotto et al. 1977
Leptodactylidae					
Eleutherodactylus rugulosus	L	1	A or "M"	Costa Rica	Tyler 1989
Leptodactylus ocellatus	$L^{E,P}$	2	M	Federative Republic of Brazil	Amaro and Sena 1968
Myobatrachidae					
Limnodynastes dumerilii	L^{P}	1	A	Australia	Tyler 1989
L. ornatus	L^{E}*	61	A, M	Australia	Tyler 1989
L. tasmaniensis	L^{E}	1	A or M	Australia	Kinghorn 1924
Neobatrachus aquilonius	L^{E}*, M*	17	A	Australia	Tyler et al. 1985
N. centralis	L^{E}*	37	A, M	Australia	Read and Tyler 1990, 1994
Notaden melanoscaphus	L^{E}	3	A or M	Australia	Tyler 1989
Uperoleia inundata	L^{E}*	9	A or M	Australia	Tyler 1989
Pelobatidae					
Pelobates cultripes	L^{P}	1	A or M	France	Gervais 1864
P. fuscus	V	2	T	France	Héron-Royer 1884
Scaphiopus bombifrons	L	6	A or M	United States	Tyler 1989
Pipidae					
Xenopus laevis	L^{P}	1	A	Republic of South Africa	Hobson 1958

Table 11-1 continued

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
Ranidae					
Rana alticola	L^E	2	M, T	India	Annandale 1905
R. arvalis	L^P	36	A, M, T	Byelorussia	Borkin and Pikulik 1986
	E, L^E, M	53	A, M	Russian Federation	Vershinin 1989, 1995b
R. aurora	$L^{E,P}$	2	A or M	United States (CA)	Cunningham 1955
R. boylii	L^P	1	A	United States (CA)	Banta 1966
R. catesbeiana	L^P	1	M	Canada (QC)	Anonymous 1944
	L^P	1	A	United States (CA)[4]	Pelgen 1951
	L^P	1	T	United States (GA)	Houck and Henderson 1953
	L^P	> 20	M	United States (OH)	Anonymous 1954
	L^P	1	M	United States (NJ)	Pearson 1960
	L^P	1	M	United States (CA)[4]	Ruth 1961
	L^P	1	A or M	United States (AL)	Anonymous 1962
	L^P	> 350	M	United States (MS)	Volpe 1970
	L^P	1	A	United States (IL)	Lopez and Maxson 1990
	O	1202	T	United States (SC)	Rowe et al. 1996
	L^E	1	M	Canada (QC)	Ouellet et al. 1997
R. chensinensis[5]	E, L^E	2384	A, M, T	Russian Federation [Sakhalin Island]	Mizgireuv et al. 1984
R. clamitans	L^P	1	A or M	United States	Duméril 1865a, 1865b
	L^P	1	M	United States (IA)	Anonymous 1945
	E, H, L^{E*}, M	> 41	A, M	United States (MI)	Martof 1956
	L^P	2	M	United States (MD)	Cooper 1958
	L^P	> 1	M	Canada (ON)	Froom 1982
	E, L^E	26	A, M	Canada (QC)	Bonin et al. 1997; Ouellet et al. 1997
R. cyanophlyctis	L^P	1	M	Sri Lanka	Deraniyagala 1944

Table 11-1 continued

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
R. esculenta complex[6]	L^P	1	A	France	Duméril 1865a, 1865b
	L^P	2	A, M	Switzerland	Lunel 1868
	L^P	1	A or M	Italy	Sordelli 1876
	L^P	2	A or M	Italy	Strobel 1876
	L^P	5	A	Italy	Ercolani 1881
	L^E	1	M	Italy	Camerano 1888
	L^P	1	M	Italy	Mazza 1888
	L^P	1	A	Russian Federation	Cholodkovsky 1896
	L^{P*}	1	M	Federal Republic of Germany	Tornier 1898
	E	1	A	France	Paris 1912
	L^P	1	T	Federal Republic of Germany	Schüßler 1925
	L^P	1	M	Federal Republic of Germany	Hellmich 1929
	L^P	21	A	France	Bonnet and Rey 1935, 1937
	L^P	1	A	Denmark	Hvass 1943
	L^P	> 1000	A, M, T	France	Rostand 1950, 1951a, 1958, 1959, 1971
	L^P	1	A	Federal Republic of Germany	Klausewitz 1952
	L^P	31	M, T	Netherlands	Hillenius 1959
	L^{P*}	1	A	Hungary	Dely 1960
	L^P	21	A, M	France	Dubois 1968
	E^*, L^{E*}	> 168	A, M, T	France [and others]	Dubois 1979
	L^P	1	M	Romania	Andrei 1985
	L^P	47	A, M	Byelorussia	Borkin and Pikulik 1986

Table 11-1 continued

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
R. graeca	L^{P}	1	M	Greece	Hellmich 1929
	L^{P}	54	M, T	Federal Republic of Yugoslavia [Montenegro]	Dubois 1974
R. grisea	L	1	A or M	[New Guinea]	Tyler 1989
R. iberica	L^{P}	3	A	Portugal	Dubois and Thireau 1972
R. lessonae	L^{P}	2	A	Latvia	Borkin and Pikulik 1986
R. nigromaculata	L^{P*}	1	A	People's Republic of China	Wu and Liu 1941
R. ornativentris	L^{P}	8	M	Japan	Takeishi 1996
R. palmipes	L^{P}	1	M	[Central or South America]	Johnson 1901
	L	1	A	Mexico (Chiapas)	Lynch 1965
R. palustris	L^{P}	1	A or M	United States (PA)	Ryder 1878
	L^{P}	1	A	United States (MA)	Tuckerman 1886
	L	12	M	United States (NC)	Murphy 1965
R. perezi	$L^{E, P*}$	4	A	Spain [Canary Islands][4]	Luis and Báez 1987
R. pipiens	L^{P}	1	A	United States (NY)	Kingsley 1881
	L^{P}	1	A	United States (IN)	Eigenmann and Cox 1901
	L^{P}	2	M	United States (IL, NY)	Johnson 1901
	L^{P}	1	A	United States (WI)	Wagner 1913
	L^{P}	1	A	United States (PA)	Colton 1922
	L^{P}	1	A	United States (MN)	Charles 1944

Table 11-1 continued

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
R. pipiens	L^{P}	3	A or M	United States (CO)	Rosine 1955
	L^{P}	1	A	United States (OH)	Adler 1958
	L^{E}	49	M	United States (MN)	Merrell 1969
	L^{E}	41	M	Canada (QC)	Ouellet et al. 1997
	$E^{*}, L^{E, P\star}$	180	M	United States (MN)	Burkhart et al. 1998
	$E^{*}, L^{E, P\star}$	> 241	M	United States (MN)	Helgen et al. 1998
R. ridibunda	L^{P}	> 546	A, M, T	Kazakhstan, Russian Federation	Woitkewitsch 1955, 1959, 1961, 1963, 1965
	L^{P}	1	A or M	Turkmenistan	Ataeva 1986
	L^{E}	4	M	Russian Federation [Ural][4]	Vershinin 1989, 1995b
	L^{E}	1471	A or M	Ukraine	Flax and Borkin 1997
R. septentrionalis	L^{P}	1	M	Canada (ON)	Anonymous 1945
R. temporaria	L^{P}	1	A	France	Duméril 1865a, 1865b
	L^{P}	1	A or M	Italy	Strobel 1876
	L^{P}	1	A	Sweden	Bergendal 1889
	L^{P}	1	A	United Kingdom	Bland Sutton 1889, 1890
	H	1	T	France	Loyez 1897

Table 11-1 continued

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
R. temporaria	L^P	1	A	[Europe]	Gemmill 1906
	L^P	1	A	United Kingdom	O'Donoghue 1910
	L^E	1	A	United Kingdom	Row 1916
	L^P	1	A	United Kingdom	Hamilton 1950
	$L^{E,P}$	> 75	A, M, T	France	Rostand 1950, 1951a, 1952a, 1956a, 1956b, 1958, 1971
	L^P	1	M	Federal Republic of Germany	Rahmann et al. 1962
	$L^{E,P}$	3	M	France	Dubois and Vachard 1969
	L^P	1	A	Federal Republic of Germany	Lüdicke 1971
	L^E	1	A	Finland	Koskela 1974
	L^P	2	A, M	Latvia	Borkin and Pikulik 1986
	L^P	1	A	Federal Republic of Germany	Meyer-Rochow and Koebke 1986
	E, L^E	11	A, M	Russian Federation	Vershinin 1989, 1995b
R. tigerina	L^P	1	A	India	Mahendra 1936

[1] According to Frost (1985), see also Schmidt (1953) and Gorham (1974) for synonymy
[2] A = Axial deformities: kyphosis, lordosis, scoliosis; E = Eye deformities: four-eyed tadpole, palatine eye, unilateral anophthalmia; H = Head deformities: absence of one tympanum, dicephalism; L = Limb deformities: anteversions, bony excrescences, bony triangles, brachydactyly, brachymely, clinodactyly, cutaneous fusion, ectopic structures, ectrodactyly (oligodactyly), ectromelia, hemimely, luxation, paralysis, permanent extension of limbs, polydactyly, polymely, polypody, retained forelimbs, subluxation, syndactyly, synmely, taumely; L^E = Mostly ectromelia and/or ectrodactyly; L^P = mostly polymely and/or polydactyly; M = Mouth deformities: injuries*, mandibular hypoplasia; O = Oral deformities: abnormal labial papillae, reduced number of labial teeth; S = Snout deformities: monorhiny; V = Spiracle deformities: abnormal development, extra spiracles; * = Some abnormalities possibly explained by traumatic events or attempted predation
[3] A = Adults: evidence of sexual maturity; M = Metamorphosing anurans: tadpoles with four limbs fully developed (Stage 42, Gosner 1960) to late juveniles (before sexual maturity); T = Tadpoles: from hatching until Stage 41 (Gosner 1960); [4] Introduced species; [5] *Rana chensinensis* = *R. pirica* for that particular locality (Matsui et al. 1993; Tanaka-Ueno et al. 1999); [6] *Rana esculenta* is a hybrid of *R. lessonae* and *R. ridibunda* (Berger 1968a, 1983)

Table 11-2 Species sensitivity and geographic distribution of published salamander deformities in the wild

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
Ambystomatidae					
Ambystoma laterale	L^{P}	11	J	Canada (NS)	Lowcock et al. 1997
A. macrodactylum	$L^{E,P}$*	1686	A, J, L	United States (CA)	Sessions and Ruth 1990
A. maculatum	L^{P}	1	A or J	United States (MA)	Kingsley 1880
	L^{P}	2	A, L	United States (MA)	Winslow 1904
	$L^{E,P}$	29	A	United States (MD, MS)	Worthington 1974
A. talpoideum	L^{P}, T	3	A	United States (SC)	Semlitsch et al. 1981
A. tigrinum	L^{P}	1	A or J	United States (MN)	Sealander 1944
	L^{P}	24	A, L	United States (CO)	Bishop 1947; Bishop and Hamilton 1947; Rosine 1955
Hynobiidae					
Salamandrella keyserlingii	E, $L^{E,P}$*	28	A, J	Russian Federation	Vershinin 1989, 1995b
Plethodontidae					
Desmognathus fuscus	T*	> 1	A	Canada (QC)	Pendlebury 1973
Plethodon albagula	$L^{E,P}$*	16	A or J	United States (TX)	Lazell 1995
P. cinereus	L^{E}*	> 12	A	Canada (NS)	Hanken 1983
	L^{E}*	> 11	A	Canada (NS), United States (ME, VA)	Hanken and Dinsmore 1986
	L^{E}*, T*	76	A, J	Canada (QC)	Bonin et al. 1999
P. glutinosus	T*	1	A	United States (MA)	Winslow 1904
	A	2	J	United States (KY)	Marvin 1995; Marvin and Hutchison 1997

Table 11-2 continued

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
P. kentucki	A, H, M	4	J	United States (KY)	Marvin and Hutchison 1997
P. neomexicanus	L^{E}*	> 4	A, J	United States (NM)	Dwyer and Hanken 1990
Salamandridae					
Chioglossa lusitanica	$L^{E, P}$*	2	A	Portugal	Dubois and Thireau 1972
Cynops orientalis	$L^{E, P}$*	12	A	People's Republic of China	Chang and Boring 1935
C. pyrrhogaster	$L^{E, P}$*, T	335	A	Japan	Meyer-Rochow and Asashima 1988
Pachytriton brevipes	$L^{E, P}$*	20	A, J	People's Republic of China	Chang and Boring 1935
Paramesotriton chinensis	$L^{E, P}$*	20	A	People's Republic of China	Chang and Boring 1935
Pleurodeles waltl	L^{P}	1	A or J	Spain	Héron-Royer 1884
Salamandra salamandra	L^{E}	1	A	United Kingdom	Howes 1893
	H, T	5	L	[Europe]	Politzer 1926
	L^{P}	1	A	Italy	Hellmich 1929
	E, L^{P}	5	L	France	Joly 1966
Taricha granulosa	$L^{E, P}$*	> 30	A	United States (CA)	Shubin et al. 1995
Triturus boscai	$L^{E, P}$*	> 9	A	Portugal	Malkmus 1981
T. carnifex	$L^{E, P}$*	> 35	A	Italy	Pacces Zaffaroni et al. 1992, 1996

Table 11-2 continued

Taxon[1]	Types of deformities[2]	No. of deformed individuals	Stage of development[3]	Country (province or state)	References
T. cristatus	L^{P}	1	A	France	Geoffroy Saint-Hilaire 1832
	L^{P}	1	A	Federal Republic of Germany	Jäckel 1881
	L^{P}	4	A	France	Dubois and Thireau 1972
T. helveticus	L^{P}	1	A	France	Rostand 1958, 1959
	L^{P}	2	A	France	Dubois and Thireau 1972
T. marmoratus	L^{P}	1	A	France	Rostand 1951a
	$L^{E, P}$*	41	A	Portugal	Caetano 1991
T. vulgaris	L^{P}	1	A	Italy	Camerano 1882
	L^{P}	1	A	Federal Republic of Germany	Landois 1884
	$L^{E, P}$*, T*	25	A	United Kingdom	Griffiths 1981
	$L^{E, P}$*	35	A	United Kingdom	Roberts and Verrell 1984
	$L^{E, P}$	11	A	Russian Federation	Vershinin 1989, 1995b

[1] According to Frost (1985), see also Schmidt (1953) and Gorham (1974) for synonymy

[2] A = Axial deformities: kyphosis, lordosis, scoliosis; E = Eye deformities: atrophy, unilateral anophthalmia; H = Head deformities: dicephalism; L = Limb deformities: abnormal webbing of toes, brachydactyly, brachymely, clinodactyly, ectopic structures, ectrodactyly (oligodactyly), ectromelia, hemimely, longer digits, polydactyly, polymely, polypody, syndactyly, synmely; L^{E} = mostly ectromelia and/or ectrodactyly; L^{P} = mostly polymely and/or polydactyly; M = Mouth deformities: mandibular hypoplasia; T = Tail deformities: amputations*, extra tails, tail projections; * = Some abnormalities possibly explained by traumatic events or attempted predation

[3] A = Adults: evidence of sexual maturity; J = Juveniles: from gill resorption for species having aquatic eggs and larvae (from hatching for species having terrestrial eggs) to late juveniles (before sexual maturity); L = Larvae: from hatching until gill resorption for species having aquatic eggs and larvae

individuals or the same sites, and others may include many species. Amphibian deformities are represented mainly by species with life histories that include aquatic eggs and larvae (indirect development). *Eleutherodactylus* frogs and *Plethodon* salamanders do not lay their eggs in water and have direct development, yet deformities have been reported in some species. The highly aquatic *Rana* species are probably overrepresented in the literature because they occur almost worldwide and have long been used in research and teaching activities. Species with a longer larval stage (e.g., up to two years in the North American *Rana catesbeiana*, *R. clamitans*, and *R. septentrionalis*) may be more susceptible if developmental perturbations are waterborne.

The most frequent amphibian deformity cited in the literature is polymely (Tables 11-1 and 11-2). However, this is probably a biased generalization, as extra limbs are spectacular and attract more attention than any other deformity (e.g., Anonymous 1964). Other types of limb abnormalities and external deformities involving the eyes, head, mouth, oral cavity, snout, and tail are also diagnosed in natural populations. The total sample size examined was not taken into consideration (Tables 11-1 and 11-2) because in reports of individual cases, it was often unknown, and in many other cases, the frequencies were not possible to break down.

Amphibian deformities do occur everywhere and in all species. Developmental abnormalities have been described in North, Central, and South America; Europe; Africa; Asia; and Australasia (Figures 11-2 and 11-3). The temperate zone is well represented by reports of mass deformities, but this may reflect more intensive investigation of these areas by herpetologists.

Possible Causes and Hypotheses

Abnormal regeneration after injury

It is well known that salamanders have the ability to regenerate lost structures throughout life, while anurans lose that power at the onset of metamorphosis. Yet the regenerative capacity in adult amphibians covers a continuum from normal regeneration to total absence of regenerative ability (Scadding 1981). Some authors (Tornier 1898; Wu and Liu 1941; Rostand 1951a; Dely 1960; Brunst 1961; Dubois 1979; Griffiths 1981) have proposed that isolated cases of polydactyly or polymely in the field may be the result of hyper-regeneration after injury. In these cases, the supernumerary digit or limb is usually heteromorphic and not symmetrical. Laboratory observations confirm this explanation. For example, a series of abnormal limbs due to conspecific bites and mutilations, and subsequent regeneration, was fully described by Duméril (1867) in captive axolotls (*Ambystoma mexicanum*). Hyper-regeneration after injury is also supported by many laboratory experiments. Surgical manipulations of limb buds during critical cell-division stages or of blastemas of regenerating limbs have been shown to be effective ways of producing

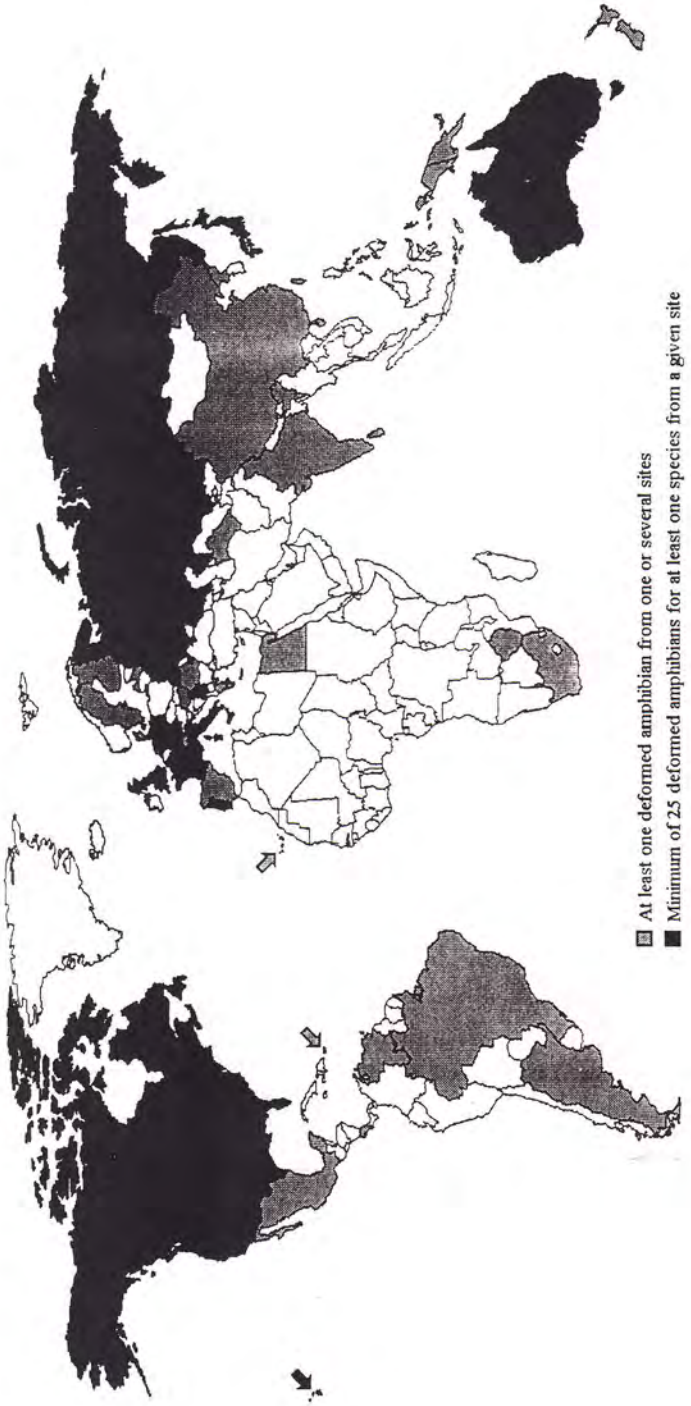

Figure 11-2 Worldwide distribution of published amphibian deformities

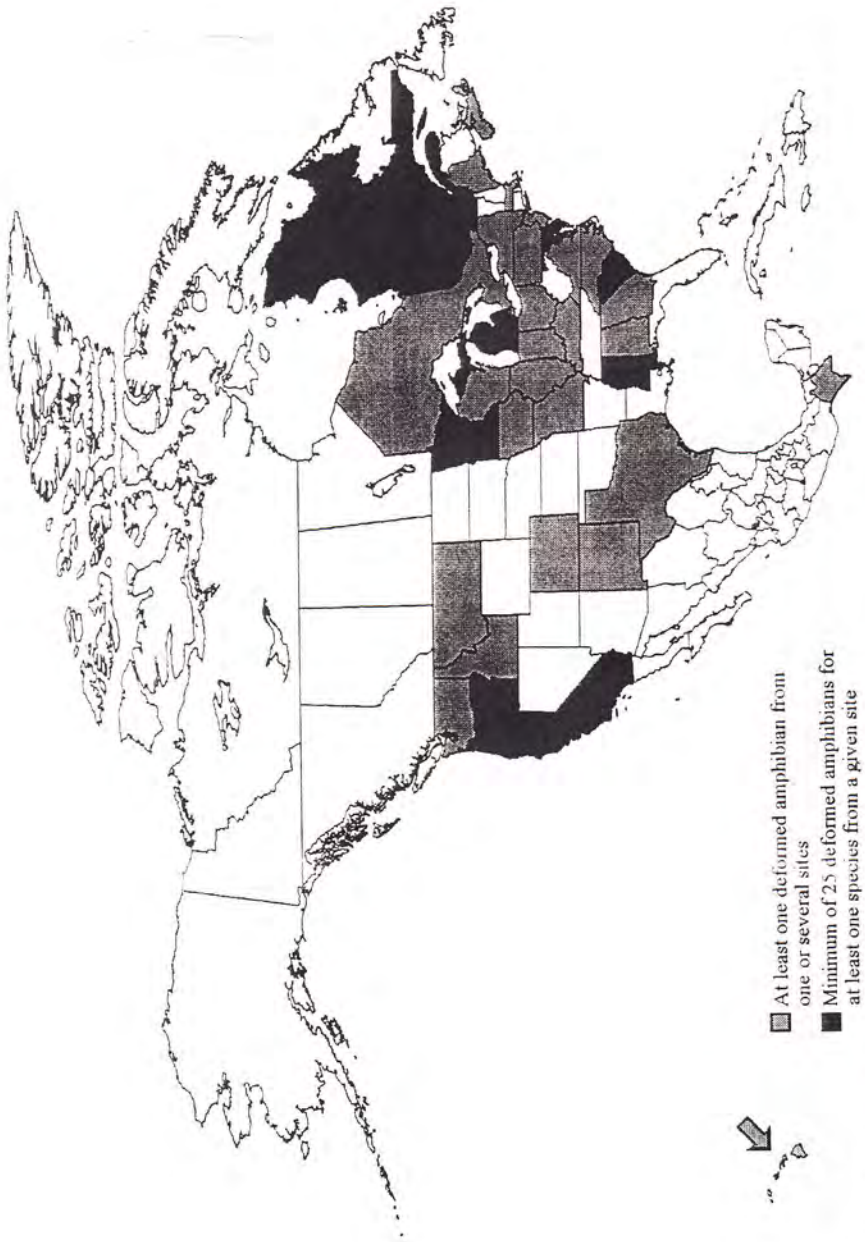

Figure 11-3 North American distribution of published amphibian deformities

extra limbs and digits in amphibians (Barfurth 1895a; Tornier 1897a, 1897b, 1905; Blount 1935; Brandt 1940; Cooper 1965; Bryant and Iten 1976; Maden 1982). More problematic to explain are cases of bilateral polymely because accidents involving both limb buds are less likely to occur in nature. Forelimb deformities are likewise difficult to explain by injury in most anurans because the forelimb buds develop protected in peribranchial sacs within the branchial chamber, whereas the hindlimb buds are in contact with the external milieu during the whole developmental process (Gosner 1960). In salamanders with aquatic larvae, the forelimbs usually undergo much of their development before hatching, while the hindlimbs develop mostly after hatching (Duellman and Trueb 1986) and are thus prone to greater exogenous assaults.

Atypical or limited limb regeneration resulting in a regenerative spike also may occur in adult amphibians following amputation (Thornton and Shields 1945; Scadding 1981) or repeated amputations (Dearlove and Dresden 1976). In the field, predatory leeches (*Erpobdella octoculata*) may damage larval hindlimbs in anurans and subsequently lead to cases of ectromelia and ectrodactyly (Veith and Viertel 1993). Hindlimb appearance after metamorphosis will depend on the level, severity, and time of injury during larval stage (Barfurth 1895b; Schotté and Harland 1943).

Agricultural pesticides and fertilizers

Agricultural herbicides, insecticides, fungicides, and fertilizers are often toxic to nontarget organisms, and can cause deformities and mortality in amphibians (Harfenist et al. 1989; Diana and Beasley 1998). In addition to the active ingredients in pesticides, a number of pesticides also contain solvents as inert ingredients that may contribute to potential developmental toxicity. Axial skeleton and tail malformations are observed after pesticide or fertilizer treatments in laboratory and field experiments conducted on anuran embryos and tadpoles (Cooke 1981; Tyler 1989; Hecnar 1995). Thus far, few studies have dealt with the full limb-development stage. Alvarez et al. (1995) reported skeletal malformations in metamorphosing *Rana perezi* kept in water containing sublethal levels of the insecticides methyl-parathion and pirimicarb. Tadpoles suffered hindlimb brachymely with twisted epiphyses of the long bones and scoliosis. The effects of the fungicide maneb (manganese ethylenebisdithiocarbamate) on limb regeneration of adult *Triturus carnifex* were examined by Arias and Zavanella (1979) and Zavanella et al. (1984). Growth retardation and skeletal abnormalities of the regenerating forelimbs were found in both studies. Brachymely, clinodactyly, ectrodactyly, syndactyly, and/or supernumerary distal bone elements were observed in all newts exposed to maneb, while only a few minor abnormalities were encountered in control animals.

Ouellet et al. (1997) reported hindlimb deformities in free-living anurans from agricultural habitats exposed to pesticide runoff in the St. Lawrence River Valley of Québec. In agricultural areas, ponds or ditches were adjacent to plots of barley, corn, potatoes, soya, sweet corn, and/or wheat. A wide range of pesticide products

was used in these sites, as often as three times during a given season. Pesticide-free control sites were localized either in pastures or old fields. Of 853 metamorphosing anurans examined in 14 farmland habitats, 106 (12.4%) had severe degrees of ectromelia and ectrodactyly, compared to only two (0.7%) of 271 in 12 unexposed sites. In this preliminary study, the variance in the proportion of deformities among sites was too great to provide statistical power sufficient to conclude that there was a significant difference between pesticide-exposed and control habitats.

Chemical composition of the water

Acidity can affect amphibians directly, as well as indirectly by influencing the toxicity of xenobiotic contaminants (Harfenist et al. 1989; see also Chapters 7 and 9, this volume). In laboratory experiments, skeletal deformities have been induced in *Rana temporaria* raised to metamorphosis at low pH (Cummins 1987, 1989). Tadpoles that grew and developed rapidly at pH 4 suffered brachymely, permanent extension, and/or grossly deformed hindlimbs. However, the effect of acidity was difficult to dissociate from tadpole crowding and from a diet of food exposed to acidified water (Cummins 1989). Further water-quality variables such as hardness, osmotic pressure, and oxygen concentration have been mentioned as potential causative agents of abnormalities, but these factors are not yet linked with actual amphibian deformities.

Coexistence with certain fishes

A severe form of polydactyly, called "Anomalie P," which is commonly accompanied with brachymely, polymely, and grossly deformed limbs, was observed over a period of two decades in some populations of the *Rana esculenta* complex in France (Rostand 1958, 1959, 1971; Dubois 1983). This condition is polymorphic but is usually bilateral and characterized by a postero-anterior gradient of teratogenicity. All the other amphibian species inhabiting these ponds were found to be physically normal. In young tadpoles exhibiting the anomaly, distal amputation of an abnormal hindlimb was followed by regeneration of a normal limb (Rostand 1952b). The coexistence of certain fishes (*Anguilla* sp., *Tinca* sp.) with *R. esculenta* during the first days of the larval life was discovered to be sufficient to induce Anomalie P. Rostand and Darré (1967, 1968) succeeded in producing abnormalities in larvae they had reared in special cages kept partly submerged in an anomaly producing pond. In these cages, two or three fishes (*Anguilla* sp., *Tinca* sp.) were restrained for two weeks with newly hatched *R. esculenta* larvae from a control area. No deformity was observed when the larvae were raised alone (Rostand et al. 1967). In the laboratory, Rostand and Darré (1969) were able to cause Anomalie P by rearing two- and three-day-old *R. esculenta* in contact with only the excrement of either of these two fish species caught in the anomaly producing pond. Larvae were considered to be sensitive to a factor present in the fishes digestive tract, the factor being perhaps a

teratogenic virus. Meanwhile, Surlève-Bazeille and Cambar (1969) were unable to induce the anomaly in *R. esculenta* when using only the mucus of these fish or bacterial cultures of it. However, cases of ectrodactyly and syndactyly were obtained by rearing larvae of *Rana temporaria* in contact with this mucus (Surlève-Bazeille, Cambar, and Mauget 1969). Histological examination of affected limbs in metamorphosing *R. esculenta* was briefly described by Surlève-Bazeille, Cambar, and Calas (1969). Light and electron microscopy revealed no viral particles or parasites in relation to limb structures (Surlève-Bazeille, Cambar, and Calas 1969; Surlève-Bazeille et al. 1970).

Diseases

Aflatoxins B_1 and G_1 diluted in water have induced teratogenic responses in *Rana temporaria* and *Bombina* sp. (Gabor et al. 1973; Puşcariu et al. 1973). Ascites was observed in tadpoles, while three metamorphosing *R. temporaria* exhibited either bilateral posterior ectromelia or hemimely.

Recent observations suggest that environmental toxicants might increase susceptibility of amphibians to disease (Carey and Bryant 1995). However, bacterial and fungal infections have not yet been incriminated in the wild as causative agents in cases of amphibian deformities. Little is known about the consequences of these infections on limb development.

Elevated tadpole densities

Berger (1968b, 1971) obtained multiple limb deformities when elevated densities of tadpoles were reared together in common aquaria. In particular, forelimb ectromelia and ectrodactyly were encountered in *Rana esculenta* complex froglets raised in densities between 3.5 to 11.1 tadpoles per liter of water. Cases of permanent extension of one or both hindlimbs also occurred (Berger 1971). It is not known if a chemical factor with some teratogenic properties can be released by crowded tadpoles.

Extreme temperatures

Anomalies in forelimb and hindlimb skeletons have been induced artificially in larvae of *Bufo vulgaris formosus* reared at a high temperature (Muto 1969a, 1969b, 1970). Digital malformations involving the metacarpal, metatarsal, and phalangeal bones (brachydactyly, ectrodactyly) were commonly observed at 30 °C, while the development was normal in control toads raised at 20 °C. The skeletal elements that differentiate at the earlier stages were more resistant to defective changes than were elements that differentiate at the later stages (Muto 1969a). Because similar anomalies were obtained at 30 °C when the water was aerated by an air pump (Muto 1971), it was determined that the high temperature was the primary teratogenic agent rather than the associated lower oxygen supply. In some laboratory stocks of *Pleurodeles waltl*, Dournon (1983) also observed that more larvae displayed hindlimb

ectrodactyly, ectromelia, or knee anteversion when reared at 30 °C compared to others kept at 20 °C.

In the field, the teratogenic action of extreme temperature has been blamed for accounts of deformities occurring in isolated habitats (Woïtkewitch 1961; Worthington 1974). Polymelous anurans were encountered in a region of cold spring water in a particular reservoir (Woïtkewitch 1961). Retardation of development in cold water might influence normal limb formation in ectotherm animals to produce deformities. Woïtkewitch (1961) also noticed that overwintering larvae of *Rana ridibunda* were prone to a higher rate of hindlimb polymely, mainly on the right body side.

Hereditary mechanisms

Some types of mass deformities may be due to genetic mutation. A recessive lethal factor was discovered in *Ambystoma mexicanum* of the mutant white strain (Humphrey 1967). The lethal trait induced limb brachymely and ectrodactyly, incomplete development of Müllerian ducts, and renal pathology and dysfunction leading to death. Droin et al. (1968) described a recessive lethal mutation that caused mandibular deformities in *Xenopus laevis* embryos. Uehlinger (1969) further reported a recessive sublethal mutation that was responsible for severe forms of polydactyly in *X. laevis*. In the same laboratory stock of *X. laevis*, cases of brachydactyly, brachymely, clinodactyly, polydactyly, and syndactyly resulted from another recessive and semilethal mutation (Droin and Fischberg 1980). These latest abnormalities were more frequent in forelimbs than in hindlimbs. A hereditary mechanism in *Bufo bufo* was also proposed by Ponse (1941) for cases of forelimb ectromelia, by Rostand (1947, 1951a) for cases of polydactyly, and by Rostand (1949a) for one case of polymely. A hereditary polymely was described in toads by Witschi and Chang (1954), but the condition was associated with egg overripeness and edema of the tadpoles. Genetic determinism has been demonstrated or hypothesized for some cases of clinodactyly, ectrodactyly, and syndactyly in *Rana temporaria* (Dubois and Vachard 1971; Dubois 1977).

Spontaneous mutations are conceivable to explain some unusual types of deformities, but this hypothesis remains generally untested. For example, abnormal webbing of toes in salamanders has been suggested by Meyer-Rochow and Asashima (1988) and Meyer-Rochow (1989) to be genetically controlled. However, this webbing might be explained by an atypical regeneration following a traumatic event.

Hybridization also has been hypothesized by Berger (1971) to explain the occurrence of some developmental abnormalities among progeny of crosses between different phenotypes of *Rana esculenta*, *R. lessonae*, and *R. ridibunda*. Spinal curvature was common in hybrid tadpoles, while permanent extension of hindlimbs and ectrodactyly were observed in metamorphosed frogs. These deformities also could be due to deficiencies in the rearing conditions. An abnormal metamorphosing

tadpole with generalized edema from a *Bombina variegata/Bombina bombina* hybridizing population from the wild was described by Gollmann et al. (1984).

Nutritional deficiencies

Skeletal malformations, luxations and subluxations of the hindlimbs, and ectrodactyly were obtained in metamorphosing *Rana perezi* reared in culture (Martínez et al. 1992). The authors suggested that the origin of the lesions was a deficiency in some compounded diets, which could alter collagen metabolism during skeletal adjustments at metamorphosis. Growing amphibians fed calcium-deficient or vitamin D-deficient foods may develop metabolic or nutritional bone disease (Crawshaw 1993; Wright 1996). Mandibular deformities, scoliosis, folding fractures of the long bones, and paralysis are sometimes observed clinically. Decreasing bone density and pathologic fractures may be seen radiographically. Diets with increased amount of calcium (Marshall et al. 1980) or vitamin C (Leibovitz et al. 1982) were both associated with a reduced incidence of scoliosis and twisted limbs in cultured *Rana catesbeiana* larvae.

The spindly leg syndrome is characterized by hypomorphic limbs, in which forelimbs often fail to emerge from the branchial chamber at metamorphosis (Crawshaw 1993; Wright 1996). A nutritional etiology also has been proposed for this condition encountered in some captive anurans, especially metamorphosing Dendrobatidae (Crawshaw 1993).

Osteolathyrogenic agents

Osteolathyrogenic defects are characterized by decreased connective tissue strength and collagen extractability, which are manifested in early embryos as notochord and tail deformities in the long axis of the animal. In metamorphosing anurans, joint dislocations and limb distortions are the clinical manifestations. Sweet pea poisoning and a variety of synthetic lathyrogenic compounds such as organic nitriles, ureides, hydrazides, and hydrazines are able to induce osteolathyrism (Levy 1959; Barrow et al. 1974). The teratogenic effects of sweet pea seeds (*Lathyrus odoratus*) and their extracts were first described in amphibians by Chang et al. (1954, 1955) on *Xenopus laevis* development and *Notophthalmus viridescens* regenerating limbs. Hindlimb deformities such as brachymely, joint dislocations, bent long bones (bony triangles), and clinodactyly were similarly obtained in *Rana temporaria* treated with *Lathyrus odoratus* seeds (Roth 1978, 1988). Toxic and osteolathyrogenic effects have also been observed in *X. laevis* embryos and metamorphosing tadpoles exposed to hydrazine, methylhydrazine, and dimethylhydrazine (Greenhouse 1976), thiosemicarbazide (Newman and Dumont 1983), semicarbazide (Schultz et al. 1985), benzoyl hydrazine (Riggin and Schultz 1986), as well as benzoic hydrazide and β-aminopropionitrile (Dawson 1993).

Parasitic cysts

The potential influence of a parasitic factor has long been suggested in the literature (Woïtkewitch 1961). Sessions and Ruth (1990) reported 205 *Hyla regilla* and 1686 *Ambystoma macrodactylum* with limb abnormalities, including supernumerary hindlimbs, in two adjacent ponds in California. Metacercarial cysts of digenetic trematodes were found preferentially localized in the cloacal and developing hindlimb regions in larvae of both species. Certain species of these parasitic flatworms use amphibians as a secondary intermediate host in a complex life cycle in which the definitive host is usually a vertebrate (e.g., aquatic birds, fishes, snakes) and pond snails are the first intermediate host. It was hypothesized by Sessions and Ruth (1990) that metacercarial cysts of trematodes can interfere mechanically with normal limb development and regeneration by disrupting positional relationships between cells to produce extra limbs in amphibians. Sessions and Ruth (1990) experimentally induced duplicated distal limb structures by implanting inert resin beads into developing limb buds of laboratory-raised *Xenopus laevis* and *Ambystoma mexicanum*. They proposed that extra hindlimbs with mirror-image duplications in the anteroposterior axis are a characteristic feature of trematode infestation (see also Sessions et al. 1999). Hindlimb deformities including cases of cutaneous fusion, ectromelia, hemimely, and polymely were recently obtained in metamorphosing *H. regilla* exposed as tadpoles to cercariae of *Ribeiroia* sp. (Johnson et al. 1999).

Digenetic trematode infestation in deformed amphibians and aquatic snail population fluctuations warrant further studies. Host animals and their parasites usually exist in relative equilibrium in most environments. Trematode communities in molluscan intermediate hosts are highly structured, very dynamic in character, and reflect long periods of coevolution (Esch and Fernandez 1994). Anthropogenic habitat alteration, introduced species, and poor water quality may influence snail dynamics, trematode abundance and interactions, and host behavior. Environmental deterioration also may compromise the immune system of amphibian hosts and affect their susceptibility to parasite infestation. Proper identification of trematode cysts following a careful dissection of deformed amphibians, and the experimental induction of limb deformities by using actual trematode cercariae remain to be performed in different amphibian species.

Radioactive pollution

Abnormalities in the formation of visual organs have been induced experimentally in *Rana nigromaculata* larvae reared in rainwater contaminated with radioactive dust (Nishimura 1967). In the wild, an outbreak of limb deformities similar to those of Anomalie P was discovered in a canal carrying waste from a nuclear research institute in the Netherlands (Hillenius 1959). The abnormalities found in metamorphosing frogs of the *Rana esculenta* complex were associated with the presence of radioactive waste. Henle (1981) reported a similar case of mass deformities in *Bufo viridis* encountered in a particular quarry in the Federal Republic of Germany. High

levels of radioactivity were first measured near the breeding pond but were not subsequently verified because of the owner's controversial destruction of the habitat. Further environmental and experimental evidence will be necessary to support this hypothesis.

Retinoids

In vertebrates, retinoids (vitamin A and derivatives) carry out important roles in cell differentiation, embryonic development, and morphogenesis. Retinoids are necessary for normal limb development but are capable of altering normal developmental pathways in certain instances (Maden 1996; Gilbert 1997). Alteration of pattern formation in both developing and regenerating amphibian limbs has been induced by exogenous vitamin A and its analogues (Niazi and Saxena 1978; Johnson and Scadding 1991; Maden 1996, 1997; Niazi 1996). A hypomorphic response such as the reduction or absence of limb structures (i.e., ectromelia and ectrodactyly) can be produced with exogenous retinoids on developing limbs. In the regenerating amphibian limb, an excess of retinoids may result in a hypermorphic response characterized by duplications of limb structures (e.g., in the proximodistal axis), which can include mirror images (Maden 1983a). The regeneration of such amputated limbs in larvae can be modified in either the proximodistal, the anteroposterior, or the dorsoventral axes (Maden 1996; Niazi 1996). Bent long bones forming bony triangles or pyramids are sometimes found in these experiments (e.g., Maden 1983a; Scadding and Maden 1986a, 1986b). Remarkably, exogenous retinoids also can cause homeotic transformation of regenerating tissue in anurans. When amputated tails of tadpoles are treated with retinoids, hindlimbs are regenerated instead of new tails (Mohanty-Hejmadi et al. 1992; Maden 1993; Mahapatra and Mohanty-Hejmadi 1994). Maden and Corcoran (1996) suggest that thyroid-hormone receptors are also involved in the homeotic transformation of tails into limbs. The retinoid effects are concentration-, time-, and stage-dependent and affect the degree of limb reduction or duplication. Of the naturally occurring retinoids, retinoic acid is the most potent (Maden 1983b), but synthetic retinoids may be more potent still.

The insecticide methoprene, a synthetic terpenoid, is an insect-growth regulator used in a variety of domestic and agricultural products. At least one metabolite of methoprene, methoprene acid, has been shown to bind to retinoid X receptors and to stimulate gene transcription in both insect and mammalian cells (Harmon et al. 1995). Methoprene is thus able to mimic the action of juvenile hormone in insects and also can activate a mammalian retinoid-responsive pathway (Harmon et al. 1995). Other synthetic ligands selective for retinoic acid receptors and retinoid X receptors have produced severe malformations in *Xenopus laevis* embryos (Minucci et al. 1996). These results raise the possibility that methoprene and other yet-to-be-discovered exogenous retinoid analogues might play a role in amphibian develop-

mental abnormalities by affecting retinoid receptor pathways. Although low methoprene concentrations may pose minimal teratogenicity for laboratory-raised amphibians (Ankley et al. 1998; La Clair et al. 1998), its metabolites and photoisomers may prove to be very teratogenic, as shown recently in *X. laevis* embryos by La Clair et al. (1998).

Teratogenic viruses

A viral etiology has been proposed, but not verified, to explain the occurrence of Anomalie P in populations of the *Rana esculenta* complex (Rostand and Darré 1969; Rostand 1971). Viruses should be considered in any investigation of abnormal amphibians, and especially in cases where diseases and mass deformities are encountered. A few viruses were recently identified in certain amphibian populations, and some were associated with episodes of mortality (Cunningham et al. 1996; Crawshaw 1997).

Trace metals

A number of metals have toxic and teratogenic effects on amphibians (Harfenist et al. 1989; Tyler 1989). Dissolved organic carbon, hardness, pH, and temperature of the water may all influence metal toxicity in breeding habitats (Freda 1991). Ocular malformations such as microphthalmia and hypopigmentation are observed in *Xenopus laevis* exposed to Ni during embryogenesis (Hauptman et al. 1993). In South Carolina, oral deformities in *Rana catesbeiana* tadpoles have been linked with coal-ash pollutants (Rowe et al. 1996, 1998). The polluted site and tadpole tissues were contaminated with a mixture of As, Ba, Cd, Cr, and Se resulting from coal combustion wastes.

Ultraviolet B radiation

Increased solar ultraviolet (UV) radiation has the potential to affect amphibian development directly, as well as indirectly through changes in water chemistry and formation of breakdown products resulting from photochemical reactions (Ovaska 1997; Blaustein et al. 1998). Cases of posterior ectromelia and polymely in *Rana temporaria* have been obtained following exposure to ultraviolet B (UV-B) radiation (Rostand 1955a, 1958). Larvae were irradiated for 15 minutes one or two days after hatching in laboratory situations. Even after prolonged exposure of larvae to ambient solar radiation, Rostand was unable to reproduce the same deformities in other experiments. Butler and Blum (1963) found that localized ultraviolet irradiation of the forelimb of larval *Ambystoma maculatum* and *A. opacum* was sufficient to cause the formation of a supernumerary limb at the level of irradiation. In the laboratory, *Bufo boreas* tadpoles exposed to enhanced levels of UV-B displayed abnormal development of the cornea, areas of hyperplasia in the integument, lordosis, and increased mortality (Worrest and Kimeldorf 1975, 1976). Lordosis in tadpoles and generalized distension in both tadpoles and newly metamorphosed

Hyla regilla and *Rana cascadae* were similarly induced under UV-B and ultraviolet A light (Hays et al. 1996). Ankley et al. (1998) have obtained many cases of hindlimb ectromelia and ectrodactyly, which usually were bilateral and often symmetrical in *Rana pipiens* held under UV light. Under field conditions, Blaustein et al. (1997) showed that *Ambystoma macrodactylum* embryos exposed to ambient levels of UV-B in controlled experiments developed edema and tail malformations.

Other xenobiotic chemicals

Various other industrial and domestic chemicals (including pharmaceutical products and endocrine disruptors) are released in aquatic ecosystems and may potentially interfere with the health of amphibian populations. Cases of hindlimb ectromelia and unilateral anophthalmia were encountered in anurans inhabiting three different regions contaminated with sewage effluent from a paper factory and municipal gutters (Mizgireuv et al. 1984). Among the 6360 *Rana chensinensis* (= *R. pirica*) examined in these regions of Sakhalin Island, Russian Federation, 2384 (37.5%) individuals of diverse ages exhibited limb abnormalities. Mizgireuv et al. (1984) also reported tumor-like dysplasia of osteochondrous tissue of hindlimbs in 328 (5.2%) of these frogs. More recently, hindlimb ectromelia and ectrodactyly, polydactyly, and tumor-like lesions were diagnosed in industrialized regions of eastern Ukraine (Flax and Borkin 1997). Of 3505 *Rana ridibunda* and 1980 *Bombina bombina* examined from water bodies polluted by factories of various types, 1471 (42.0%) and 567 (28.6%) individuals, respectively, showed limb abnormalities. In the Urals, greater percentages of morphological deformities were observed in heavily urbanized areas than in more natural habitats (Vershinin 1989, 1995a, 1995b). Environmental pollution in urban regions could be responsible for the higher rates of developmental deformities, abnormal regeneration, mutation frequencies, and neoplasms in both frogs and salamanders.

Implications in Amphibian Population Declines

In areas where deformities were abundant in the 1950s and 1960s, anuran survivorship was already a matter of concern (Rostand and Darré 1968; Rostand 1971). Severe cases of Anomalie P were always lethal under field conditions at the metamorphic stage. Injuries and mechanical interferences caused by the abnormal hindlimbs were responsible for the mortality (Rostand 1955b). The highest prevalences of amphibian deformities are found in metamorphosing individuals while low frequencies prevail among adults (Martof 1956; Rostand 1959; Sessions and Ruth 1990; Veith and Viertel 1993; Ouellet et al. 1997; Johnson et al. 1999). It is easy to see that developmental abnormalities are maladaptive and can affect survivorship by interfering with swimming, hopping, acquisition of food, and avoidance of predators. Abnormalities are likely to affect population recruitment at local and regional scales. Thus far, deformities seem site-specific, and there is no real

evidence of an unitary global phenomenon. Since one or more of a variety of factors may cause amphibian deformities, population level studies will be required to determine the extent to which mass deformities contribute to amphibian declines at local, regional, or global scales.

Environmental Degradation and Human-health Concerns

In humans, deformities very similar to those encountered in amphibians are not rare and have long been reported in the scientific literature (Liceti 1634; Vallisneri 1733a, 1733b, 1733c; De Superville 1740; Virey 1819; Geoffroy Saint-Hilaire 1832, 1836a, 1836b; Handyside 1866; Bateson 1894). More recently, Schwartz and LoGerfo (1988) associated congenital limb reduction defects with parental involvement in agricultural work and residence in agricultural settings. The authors stressed that although pesticides are the most obvious toxic agent, other environmental exposures are unique to the agricultural setting, such as inorganic and organic fertilizers and specific pollens. Garry et al. (1996) reported that private pesticide appliers and families residing in predominantly agricultural regions are more likely to have children with birth anomalies. Excess frequencies of musculoskeletal anomalies including limb reduction defects, polydactyly, syndactyly, and adactyly were associated with agricultural work by pesticide appliers. A recent literature review also reported that there were some indications of elevated risks of limb anomalies and orofacial clefts associated with environmental or occupational exposure to pesticides (Nurminen 1995).

Today, large numbers of pesticides and industrial chemicals are widely distributed in the environment. These pollutants have multiple modes of action, and many can disturb normal development and endocrine function in both wildlife and humans (Zile 1992; Colborn et al. 1993; Guillette 1995; Hayes 1997; LeBlanc and Bain 1997; Arbuckle and Sever 1998; Cheek et al. 1998). Endocrine-disrupting toxicants may have effects at cellular and tissue levels well below detectable levels.

Clinical examination and measurement of frequency of external deformities in tadpoles (Cooke 1981) and metamorphosing anurans (Ouellet et al. 1997) have been proposed as a first step to the assessment of ecosystem health and the detection of environmental contaminants in agricultural habitats. This screening tool is economical and noninvasive and may be of ecological significance, especially as an indicator in freshwater ecosystems. The relevance to human health lies in the understanding of cause-effect linkages between all possible natural and anthropogenic factors and the high frequencies of amphibian deformities where they occur. Consideration should be given to the fact that environmental pollutants might produce a broad spectrum of sublethal and lethal effects that are still poorly understood. Deformity rates are determined from survivors and, if sublethal

responses to some contaminants are skewed toward lethality, their impact might be underestimated. In farming regions of the St. Lawrence River Valley of Québec, genotoxic effects, assayed as DNA profile abnormalities, were identified by flow cytometry in outwardly deformed metamorphosing *Rana clamitans* as well as in apparently healthy adults where both were subjected to pesticide applications (Bonin et al. 1997; Lowcock et al. 1997). As another example, Honrubia et al. (1993) showed that chronic exposure to pirimicarb, a carbamate insecticide, induced structural changes of gills, liver, gallbladder, heart, and notochord in *Rana perezi* tadpoles. Physical deformities may initially be noticed, but they may represent only one of several developmental, or more subtle, endpoints.

Recommendations and Suggestions for Future Research

Recent observations of high frequencies of mass deformities in amphibians (Mizgireuv et al. 1984; Flindt 1985; Vershinin 1989, 1995b; Sessions and Ruth 1990; Veith and Viertel 1993; Flax and Borkin 1997; Ouellet et al. 1997; Burkhart et al. 1998; Helgen et al. 1998; Johnson et al. 1999) clearly deserve further investigation. Amphibian deformity surveys should be standardized to enable statistical comparisons and to allow inferences regarding the possible causes. Deformity distribution data should be incorporated into computer-based geographic information systems to analyze spatially related land-use patterns and environmental factors. Abnormalities need to be described with proper technical terms (see Appendix) and categorized because more than one phenomenon may occur simultaneously.

Although sometimes difficult, it is important to differentiate between traumatic injuries (e.g., digit amputation, eye enucleation) and true developmental abnormalities. Attempted predation, mechanical accidents, conspecific fighting, and sequelae from a previous disease are all potential factors that may cause injuries in amphibians. Leeches attached to digit extremities and small bivalve molluscs clenching the tips of toes have been noticed to cause digit injuries in *Rana* and *Triturus* species (Dubois 1979). Breeding *Ambystoma laterale* were observed transporting pea clams (*Pisidium adamsi*) attached to digits of their hindlimbs, causing swelling at the point of attachment (Davis and Gilhen 1982). Abrasion injuries of digits and sides of the mouth were recorded in 17 adult *Neobatrachus aquilonius* found in an empty swimming pool (Tyler et al. 1985). The self-inflicted injuries exhibited by this fossorial species were consistent with the frogs' attempting to burrow into the concrete floor. Bleeding or a red appearance of cutaneous surfaces, healing wounds of superficial soft tissues and/or bones, scar tissue, and stumps with or without cartilaginous regenerative spikes are common features diagnostic of trauma. Unilateral and asymmetrical lesions may be more indicative of traumatic instances, in contrast to the bilateral and symmetrical lesions that are more likely developmental defects. Number of individuals, age group, species, and number of species involved are also important considerations that can help pinpoint a cause. In

comparison with anurans, limb and eye abnormalities in newts and salamanders are more difficult to classify because of the regenerative capacity of these animals (Scadding 1981; Mitashov 1997). However, both a traumatic injury during tadpole development in anurans or throughout life in salamanders may stimulate an abnormal regenerative response and generate a developmental abnormality. For example, a young newt may grow an extra foot, a developmental abnormality, from a complicated wound resulting from attempted predation or a traumatic injury.

Radiographic examination is a noninvasive approach that can be used in amphibians to better diagnose doubtful cases and can reveal, for example, an old fracture causing limb asymmetry or duplicated internal limb structures. High resolution radiography may be useful to detect skeletal and other internal abnormalities. Clearing and differential staining techniques for bone and cartilage (Wassersug 1976; Hanken and Wassersug 1981) can be used on freshly dead or voucher specimens to characterize deformities and search for metacercarial cysts in relation to limb structures (Sessions and Ruth 1990). In these procedures, cartilage will appear blue when stained with Alcian Blue, and bone will stain red with Alizarin Red S. Soft tissue will become translucent or transparent. An automated double-staining protocol using Alcian Blue for cartilage and Murexide for bone has also been developed (Miller and Tarpley 1996). Another whole-mount technique using Victoria Blue B is described by Bryant and Iten (1974). Patterns of skin pigmentation should be noted on abnormal limb structures before using such techniques. Histopathology is another postmortem diagnostic tool that may shed light on the nature of abnormalities.

Properly designed sampling methods are crucial to avoid bias and misrepresentation of current frequencies of amphibian deformities. Anuran surveys should focus on the metamorphosing age group. The prevalence of deformities for the same age group of the same species may vary between years at a given site. For example, frequencies ranged from 0 to 80% during a multi-year survey of *Rana esculenta* complex in a particular French site (Rostand 1971), and from 0 to 66% in a two-year survey of *Rana pipiens* in a Québec pond (Ouellet et al. 1997). In contrast, annual variation in the frequency of limb abnormalities was not high in some polluted areas of Ukraine (Flax and Borkin 1997). During a given season, the month and even the day of collection may cause discrepancies between results for the same habitat. Merrell (1969) found that in a Minnesota lake 22.5% of *R. pipiens* were deformed at the end of July; two days later, the rate was 8.4%, then 14.2% in mid-August, and finally 3.6% in late September. Similarly, the frequency of deformed *R. esculenta* in a French pond was 2% in May, and 20% for both a June and a July collection (Rostand 1971). Furthermore, Rostand (1971) noted that deformity rates were variable between the different areas of the same pond and that these regions of high deformity rates moved from year to year.

The Frog Embryo Teratogenesis Assay - *Xenopus* (FETAX) is a 96-h whole-embryo developmental toxicity screening assay used in ecotoxicology that utilizes the

laboratory-raised embryos of *X. laevis* (ASTM 1991; Bantle et al. 1991; Bantle 1995). As a first step, FETAX is well suited for testing chemicals and environmental samples. Even though a large genetic distance separates laboratory *X. laevis* and native temperate species, developmental pathways are highly conserved. However, the FETAX assay should be expanded to include the full limb-development stage. The standard 96-h assay has been used by Burkhart et al. (1998) to evaluate the capacity of water samples from Minnesota to induce malformations in embryos of *X. laevis*. Using water from ponds with high incidences of frog malformations, Burkhart et al. (1998) suggest that water in the affected sites contains one or more unknown agents that induce developmental abnormalities and mortality in *X. laevis*.

Controlled experiments under field conditions with native amphibian species (e.g., *Rana* sp.) need to be performed to investigate the ambient levels of environmental factors as potential causes of amphibian deformities. Rearing conditions (e.g., tadpole densities, ion concentrations, metals, pH) are crucial in such experiments. Even in the best control situations, amphibian deformities may occur spontaneously (Reichenow 1908; Woodland 1908; Fischer 1971, 1973, 1977; Lauthier 1971; Dubois and Fischer 1975; Cooke 1981; Greer 1997). Overripeness of the eggs has been incriminated as a cause of teratogenesis (Witschi 1952), but Rostand (1951b) was unable to obtain more limb anomalies with overripe eggs than with normal ones.

Caution is warranted if more effort is to be undertaken to establish the distribution and prevalence of amphibian deformities. Excessive and unnecessary sampling may be detrimental to amphibian populations and may disturb sensitive aquatic ecosystems. Field investigators also have the potential to act as vectors of infectious diseases and parasites. No single cause will likely explain mass occurrences of amphibian deformities in the wild. The causes are probably local or regional, and a complex interaction of multiple factors is also conceivable. Diagnosis may be further complicated due to possible time lag between the presence of a factor and later detection of deformities. Trauma, parasitic trematode infestation, and xenobiotic pesticides or chemicals (perhaps affecting retinoid receptor pathways), along with a synergistic action of UV-B radiation, emerge as the leading hypotheses. Other yet undiscovered causative agents may be at work too.

Acknowledgments—I thank Heather Gray, David M. Green, Linda Paetow, and Marc Trudel from McGill University for helpful comments during the preparation of this literature review. I also acknowledge Jocelyn Ouellet for assistance with the maps. I was supported, in part, by a FCAR Québec scholarship and a NSERC Canada research grant to David M. Green.

Appendix to Chapter 11

Glossary

Anomalie P: severe form of polydactyly commonly accompanied with brachymely, polymely, and grossly deformed limbs (see Rostand 1958, 1959, 1971)

Anteversion: a joint oriented in a forward direction (e.g., a knee anteversion)

Ascites: accumulation of serous fluid in the peritoneal cavity (abdominal edema)

Bony triangle: bent long bone(s) forming a bony triangle or pyramid

Brachydactyly: shorter digit(s)

Brachymely: shorter limb(s)

Clinodactyly: curvature of one or more digits

Dicephalism: two heads

Ectrodactyly (oligodactyly): absence of one or more digits (*adactyly*) or parts of digits

Ectromelia: absence of one or more limbs or parts of limbs

Hemimely: absence of all or part of the distal half of a limb

Kyphosis: abnormal backward curvature of the spine

Lordosis: abnormal forward curvature of the spine

Luxation: dislocation of a joint

Mandibular hypoplasia: underdeveloped mandible

Microphthalmia: eye smaller than normal

Monorhiny: having a single nostril

Palatine eye: eye in mouth

Polydactyly: supernumerary digit(s)

Polymely: supernumerary limb(s)

Polypody: a limb with two or more hands or feet

Scoliosis: abnormal lateral curvature of the spine

Subluxation: incomplete or partial dislocation of a joint

Syndactyly: fusion of two or more digits

Synmely: fusion of a limb or parts of a limb to a body part

Synrhiny: joined nostrils

Taumely: a limb element oriented at 90° to the long axis of the limb

Unilateral anophthalmia: absence of one eye

References

Adler KK. 1958. A five-legged *Rana* from Ohio. *Ohio Herpetol Soc Trimon Rep* 1:21.

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

Alvarez R, Honrubia MP, Herráez MP. 1995. Skeletal malformations induced by the insecticides ZZ-Aphox® and Folidol® during larval development of *Rana perezi*. *Arch Environ Contam Toxicol* 28:349–356.

Amaro A, Sena S. 1968. Ocorrências de anomalias em anfíbios. *Atas Soc Biol Rio de Janeiro* 12:95–96.

Andrei M. 1985. Un cas de polymélie dans le complexe *Rana "esculenta"* Linné (Anura). *Trav Mus Hist Nat "Grigore Antipa"* (27):267–268.

Ankley GT, Tietge JE, DeFoe DL, Jensen KM, Holcombe GW, Durhan EJ, Diamond SA. 1998. Effects of ultraviolet light and methoprene on survival and development of *Rana pipiens*. *Environ Toxicol Chem* 17:2530–2542.

Annandale N. 1905. On abnormal ranid larvae from north-eastern India. *Proc Zool Soc Lond* 1:58–61.

[Anonymous]. 1944. Another abnormal frog. *Turtox News* 22:183.

[Anonymous]. 1945. More many-legged frogs. *Turtox News* 23:86–87.

[Anonymous]. 1954. Many-legged frogs. *Sci News Let* 66:327.

[Anonymous]. 1962. Alabama biologist has 12-legged bullfrog. *Bull Philadelphia Herpetol Soc* 10(1):24–25.

[Anonymous]. 1964. Frogs with 5 legs and more found in pond in Jersey. *NY Times* 113(Sept 5):21.

Arbuckle TE, Sever LE. 1998. Pesticide exposures and fetal death: a review of the epidemiologic literature. *Crit Rev Toxicol* 28:229–270.

Arias E, Zavanella T. 1979. Teratogenic effects of manganese ethylenebisdithiocarbamate (maneb) on forelimb regeneration in the adult newt, *Triturus cristatus carnifex*. *Bull Environ Contam Toxicol* 22:297–304.

[ASTM] American Society for Testing and Materials. 1991. Standard guide for conducting the Frog Embryo Teratogenesis Assay - *Xenopus* (FETAX). Philadelphia PA: American Society for Testing and Materials. ASTM Designation E 1439–91. 11 p.

Ataeva AA. 1986. A case of five-legged *Rana ridibunda* from water bodies of Turkmenistan. *Izvest Akad Nauk TurkmSSR, Ser Biol Nauk* (4):78. [In Russian].

Banta BH. 1966. A six-legged anuran from California. *Wasmann J Biol* 24:67–69.

Bantle JA. 1995. FETAX - A developmental toxicity assay using frog embryos. In: Rand GM, editor. Fundamentals of aquatic toxicology. 2nd edition. Washington DC: Taylor and Francis. p 207–230.

Bantle JA, Dumont JN, Finch RA, Linder G. 1991. Atlas of abnormalities: a guide for the performance of FETAX. Stillwater OK: Oklahoma State Publications Department. 68 p.

Barfurth D. 1895a. Die experimentelle regeneration überschüssiger gliedmaßentheile (polydaktylie) bei den amphibien. *Arch Entwicklungsmech Organ* 1:91–116.

Barfurth D. 1895b. Sind die extremitäten der frösche regenerationsfähig? *Arch Entwicklungsmech Organ* 1:117–123.

Barrow MV, Simpson CF, Miller EJ. 1974. Lathyrism: a review. *Quart Rev Biol* 49:101–128.

Bateson W. 1894. Materials for the study of variation. London, UK: Macmillan. 598 p.

Bergendal D. 1889. Über eine dritte vordere extremität eines braunen frosches. *Bihang Kongl Svensk Vet Akad Handl* 14(IV)(8):1–35.

Berger L. 1968a. Morphology of the F_1 generation of various crosses within *Rana esculenta*-complex. *Acta Zool Cracov* 13:301–324.

Berger L. 1968b. The effect of inhibitory agents in the development of green-frog tadpoles. *Zool Polon* 18:381–390.

Berger L. 1971. Viability, sex and morphology of F_2 generation within forms of *Rana esculenta* complex. *Zool Polon* 21:345–393.

Berger L. 1983. Western palearctic water frogs (Amphibia, Ranidae): systematics, genetics and population compositions. *Experientia* 39:127–130.

Bishop DW. 1947. Polydactyly in the tiger salamander. *J Hered* 38:290–293.

Bishop DW, Hamilton R. 1947. Polydactyly and limb duplication occurring naturally in the tiger salamander, *Ambystoma tigrinum*. *Science* 106:641–642.

Bland Sutton J. 1889. Supernumerary limbs in frogs and toads. *Trans Pathol Soc* 40:461–463.

Bland Sutton J. 1890. Evolution and disease. New York NY: Scribner and Welford. 285 p.

Blaustein AR, Kiesecker JM, Chivers DP, Anthony RG. 1997. Ambient UV-B radiation causes deformities in amphibian embryos. *Proc Natl Acad Sci* 94:13735–13737.

Blaustein AR, Kiesecker JM, Chivers DP, Hokit DG, Marco A, Belden LK, Hatch A. 1998. Effects of ultraviolet radiation on amphibians: field experiments. *Am Zool* 38:799–812.

Blount IWH. 1935. The anatomy of normal and reduplicated limbs in Amphibia, with special reference to musculature and vascularization. *J Exp Zool* 69:407–457.

Bohl E. 1997. Mißbildungen der extremitäten von amphibienlarven in Aufseß (Oberfranken): versuche zur ursachenermittlung. *Münch Beitr Abwass Fisch Flussbiol* 50:160–189.

Bonin J, Desroches J-F, Ouellet M, Leduc A. 1999. Les forêts anciennes: refuges pour les salamandres. *Nat Can* 123:13–18.

Bonin J, Ouellet M, Rodrigue J, DesGranges J-L, Gagné F, Sharbel TF, Lowcock LA. 1997. Measuring the health of frogs in agricultural habitats subjected to pesticides. In: Green DM, editor. Amphibians in decline: Canadian studies of a global problem. Volume 1, Herpetological conservation. Saint Louis MO: Society for the Study of Amphibians and Reptiles. p 246–257.

Bonnet A, Rey M. 1935. Sur quelques monstruosités présentées par la grenouille. *Bull Soc Zool Fr* 60:338–341.

Bonnet A, Rey M. 1937. Sur quelques cas de polydactylie et de schistodactylie observés en série chez la grenouille. *Bull Soc Zool Fr* 62:21–25.

Borkin LJ, Pikulik MM. 1986. The occurrence of polymely and polydactyly in natural populations of anurans of the USSR. *Amphib Rept* 7:205–216.

Brandt W. 1940. Experimental production of functioning reduplications - a triple and a functioning quintuple hindlimb in the frog. *J Exp Biol* 17:396–401.

Brunst VV. 1961. Some problems of regeneration. *Quart Rev Biol* 36:178–206.

Bryant SV, Iten LE. 1974. The regulative ability of the limb regeneration blastema of *Notophthalmus viridescens*: experiments in situ. *Wilhelm Roux' Arch* 174:90–101.

Bryant SV, Iten LE. 1976. Supernumerary limbs in amphibians: experimental production in *Notophthalmus viridescens* and a new interpretation of their formation. *Dev Biol* 50:212–234.

Burkhart JG, Helgen JC, Fort DJ, Gallagher K, Bowers D, Propst TL, Gernes M, Magner J, Shelby MD, 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.

Butler EG, Blum HF. 1963. Supernumerary limbs of urodele larvae resulting from localized ultraviolet light. *Dev Biol* 7:218–233.

Caetano MH. 1991. Anomalies et régénération des membres chez *Triturus marmoratus* (Latreille, 1800). *Bull Soc Herpétol Fr* (57):53–58.

Camerano L. 1882. Di un caso di polimelia in un *Triton taeniatus* (Schneid.). *Atti Soc Ital Sci Nat* 25:113–116.

Camerano L. 1888. Descrizione di un girino anomalo di *Rana esculenta* Linneo. *Boll Mus Zool Anat Comp R Univ Torino* 3(36):1–2.

Carey C, Bryant CJ. 1995. Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. *Environ Health Perspect* 103(Suppl 4):13–17.

Chan JG, Young LL. 1985. *Bufo marinus* (marine toad): anomaly. *Herpetol Rev* 16:23–24.

Chang CY, Witschi E, Ponseti IV. 1954. Teratologic development in *Xenopus* larvae caused by sweet pea seeds (*Lathyrus odoratus*) and their extracts. *Anat Rec* 120:816.

Chang CY, Witschi E, Ponseti IV. 1955. Teratogenic effects of *Lathyrus odoratus* seeds on development and regeneration of vertebrate limbs. *Proc Soc Exp Biol Med* 90:45–50.

Chang T-K, Boring AM. 1935. Studies in variation among the Chinese Amphibia. I. Salamandridae. *Peking Nat Hist Bull* 9:327–360.

Charles H. 1944. Abnormal frog. *Turtox News* 22:179.

Cheek AO, Vonier PM, Oberdörster E, Burow BC, McLachlan JA. 1998. Environmental signaling: a biological context for endocrine disruption. *Environ Health Perspect* 106(Suppl 1):5–10.

Cholodkovsky N. 1896. Sur quelques exemples de polydactylie. *C R Trav Soc Imp Nat St Pétersbourg* 27:74–80. [In Russian].

Cisternas R. 1865. Mélanges et nouvelles. *Rev Mag Zool* (Sér 2) 17:287–288.

Colborn T, vom Saal FS, Soto AM. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ Health Perspect* 101:378–384.

Colton HS. 1922. The anatomy of a five legged frog. *Anat Rec* 24:247–253.

Cooke AS. 1981. Tadpoles as indicators of harmful levels of pollution in the field. *Environ Pollut* (Ser A) 25:123–133.

Cooper EL. 1965. Abnormal limb development in *Rana catesbeiana*. *Proc Soc Exp Biol Med* 118:201–204.

Cooper JE. 1958. Some albino reptiles and polydactylous frogs. *Herpetologica* 14:54–56.

Crawshaw GJ. 1993. Amphibian medicine. In: Fowler ME, editor. Zoo and wild animal medicine. Current therapy III. Philadelphia PA: WB Saunders. p 131–139.

Crawshaw GJ. 1997. Disease in Canadian amphibian populations. In: Green DM, editor. Amphibians in decline: Canadian studies of a global problem. Volume 1, Herpetological conservation. Saint Louis MO: Society for the Study of Amphibians and Reptiles. p 258–270.

Crosswhite E, Wyman M. 1920. [No title]. *J Entomol Zool* 12:78.

Cummins CP. 1987. Factors influencing the occurrence of limb deformities in common frog tadpoles raised at low pH. *Ann Soc R Zool Belg* 117(Suppl 1):353–364.

Cummins CP. 1989. Interaction between the effects of pH and density on growth and development in *Rana temporaria* L. tadpoles. *Func Ecol* 3:45–52.

Cunningham AA, Langton TES, Bennett PM, Lewin JF, Drury SEN, Gough RE, MacGregor SK. 1996. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). *Philos Trans R Soc Lond* (B)351:1539–1557.

Cunningham JD. 1955. Notes on abnormal *Rana aurora draytoni*. *Herpetologica* 11:149.

Davis DS, Gilhen J. 1982. An observation of the transportation of pea clams, *Pisidium adamsi*, by blue-spotted salamanders, *Ambystoma laterale*. *Can Field-Nat* 96:213–215.

Dawson DA. 1993. Joint action of benzoic hydrazide and ß-aminopropionitrile on *Xenopus* embryo development. *Toxicology* 81:123–130.

Dearlove GE, Dresden MH. 1976. Regenerative abnormalities in *Notophthalmus viridescens* induced by repeated amputations. *J Exp Zool* 196:251–261.

Dely OG. 1960. Une grenouille verte (*Rana esculenta* L.) à cinq extrémités. *Vertebr Hungarica* 2:41–47.

Deraniyagala PEP. 1944. A teratological frog from Ceylon. *J Ceylon Branch R Asiatic Soc* 36:224–225.

De Superville D. 1740. Some reflections on generation, and on monsters, with a description of some particular monsters. *Philos Trans* 41:294–307.

Diana SG, Beasley VR. 1998. Amphibian toxicology. In: Lannoo MJ, editor. Status and conservation of Midwestern amphibians. Iowa City IA: University of Iowa. p 266–277.

Dournon C. 1983. Développement somatique et inversion sexuelle sous l'action de la température chez les amphibiens. In: Vago C, Matz G, editors. Comptes Rendus du Premier Colloque International de Pathologie des Reptiles et des Amphibiens. Angers, France: Presses de l'Université d'Angers. p 227–231.

Droin A, Fischberg M. 1980. Abnormal limbs (abl), a recessive mutation affecting the tadpoles of *Xenopus l. laevis*. *Experientia* 36:1286–1288.

Droin A, Reynaud J, Uehlinger V. 1968. Folded jaw (fj), une mutation létale récessive affectant le développement de la mâchoire chez *Xenopus laevis*. *Rev Suisse Zool* 75:531–538.

Dubois A. 1968. Sur deux anomalies de la grenouille verte (*Rana esculenta*). *Bull Soc Linn Lyon* 37:316–320.

Dubois A. 1974. Polydactylie massive, associée à la clinodactylie, dans une population de *Rana graeca*. Remarques sur la polydactylie faible et la clinodactylie chez *Bufo bufo*. (Amphibiens, Anoures). *Bull Soc Zool Fr* 99:505–521.

Dubois A. 1977. Une mutation dominante déterminant l'apparition de diverses anomalies digitales chez *Rana temporaria* (Amphibiens, Anoures). *Bull Soc Zool Fr* 102:197–213.

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

Dubois A. 1983. L'anomalie P des grenouilles vertes (complexe de *Rana* kl. *esculenta* Linné, 1758) et les anomalies voisines chez les amphibiens. In: Vago C, Matz G, editors. Comptes Rendus du Premier Colloque International de Pathologie des Reptiles et des Amphibiens. Angers, France: Presses de l'Université d'Angers. p 215–221.

Dubois A, Fischer J-L. 1975. Un leptodactyle pentadactyle ectrodactyle (Amphibiens, Anoures). *Bull Soc Linn Lyon* 44:111–114.

Dubois A, Thireau M. 1972. Polydactylie chez *Rana iberica* Boulenger, 1879 (Amphibiens, Anoures). *Bull Mus Natl Hist Nat, Zool* 22:157–162.

Dubois A, Vachard D. 1969. Sur trois anomalies digitales de la grenouille rousse (*Rana temporaria*). *C R Soc Biol* 163:2255–2257.

Dubois A, Vachard D. 1971. Sur la descendance d'une grenouille rousse (*Rana temporaria*) ectrodactyle. *C R Soc Biol* 165:26–29.

Duellman WE, Trueb L. 1986. Biology of amphibians. New York NY: McGraw-Hill. 670 p.

Duméril A. 1865a. Observations sur la monstruosité dite polymélie ou augmentation du nombre des membres chez des batraciens anoures. *Nouv Arch Mus Hist Nat* 1:309–319.

Duméril A. 1865b. Trois cas de polymélie (membres surnuméraires) observés sur des batraciens du genre *Rana*. *C R Acad Sci* 60:911–913.

Duméril A. 1867. Description de diverses monstruosités observées à la Ménagerie des Reptiles du Muséum d'Histoire Naturelle sur les batraciens urodèles à branchies extérieures dits axolotls. *Nouv Arch Mus Hist Nat* 3:119–130.

Dunayev EA. 1997. A record of the green toad (*Bufo viridis*) with five legs in Moscow Province. *Adv Amphib Res Former Soviet Union* 2:169–171.

Dwyer CM, Hanken J. 1990. Limb skeletal variation in the Jemez Mountains salamander, *Plethodon neomexicanus*. *Can J Zool* 68:1281–1287.

Eigenmann CH, Cox UO. 1901. Some cases of saltatory variation. *Am Nat* 35:33–38.

Ercolani GB. 1881. Della polidactylia, della melomelia e della polimelia nei rettili e di alcune forme di mostruosità con queste corrispondenti, osservate negli organi della locomozione nei pesci. *Mem Accad Sci Ist Bologna* (4)3:810–824.

Esch GW, Fernandez JC. 1994. Snail-trematode interactions and parasite community dynamics in aquatic systems: a review. *Am Midl Nat* 131:209–237.

Fischer J-L. 1971. Luxation et raideur permanente des membres postérieurs chez la grenouille rousse, *Rana temporaria*. *Bull Soc Linn Lyon* 40:292–294.

Fischer J-L. 1973. Polydactylie faible chez la grenouille rousse. *Bull Soc Linn Lyon* 42:1–4.

Fischer J-L. 1977. Un mime morphologique de la polydactylie faible: la fissuration de phalanges distales chez *Rana temporaria* (Amphibiens, Anoures). *Bull Soc Linn Lyon* 46:143–146.

Flax NL, Borkin LJ. 1997. High incidence of abnormalities in anurans in contaminated industrial areas (eastern Ukraine). In: Böhme W, Bischoff W, Ziegler T, editors. Herpetologia bonnensis. Bonn, Fed Repub Ger: SEH. p 119–123.

Flindt R. 1985. Untersuchungen zum auftreten von mißgebildeten wechselkröten (*Bufo viridis*) in einem steinbruch in Vaihingen-Roßwag. *Jahresh Ges Naturk Württemb* 140:213–233.

Freda J. 1991. The effects of aluminum and other metals on amphibians. *Environ Pollut* 71:305–328.

Froom B. 1982. Amphibians of Canada. Toronto, Ontario, Canada: McClelland and Stewart. 120 p.

Frost DR, editor. 1985. Amphibian species of the world. Lawrence KS: Allen Press and ASC. 732 p.

Gabor M, Puşcariu F, Deac C. 1973. Action tératogène des aflatoxines sur les têtards. *Arch Roum Pathol Exp Microbiol* 32:269–275.

Gaggero P. 1960. Caso de monstruosidad en el sapo *Bufo arenarum* Hensel. *Actas Trab Prim Congr Sudam Zool* 4:69–72.

Garry VF, Schreinemachers D, Harkins ME, Griffith J. 1996. Pesticide appliers, biocides, and birth defects in rural Minnesota. *Environ Health Perspect* 104:394–399.

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

Geoffroy Saint-Hilaire I. 1832. Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux. Volume 1. Paris, France: Baillière. 746 p.

Geoffroy Saint-Hilaire I. 1836a. Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux. Volume 2. Paris, France: Baillière. 571 p.

Geoffroy Saint-Hilaire I. 1836b. Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux. Volume 3. Paris, France: Baillière. 618 p.

Gervais P. 1864. Cas de polymélie (membres surnuméraires) observés sur un batracien du genre *Pelobates* et sur une espèce du genre *Raie*. *C R Acad Sci* 59:800–802.

Ghorab HM. 1959. On the occurrence of an accessory fore-limb in the Egyptian maculated toad, *Bufo regularis* Reuss. *A'In Shams Sci Bull* (4):109–113.

Gilbert SF. 1997. Developmental biology. 5th edition. Sunderland MA: Sinauer Associates. 918 p.

Gollmann G, Hödl W, Ohler A. 1984. A tadpole from a *Bombina* hybrid population - a hopeless monster. *Amphib Rept* 5:411–413.

Gorham SW. 1974. Checklist of world amphibians up to January 1, 1970. Saint John, New Brunswick, Canada: New Brunswick Museum. 173 p.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.

Green DM, editor. 1997. Amphibians in decline: Canadian studies of a global problem. Volume 1, Herpetological conservation. Saint Louis MO: Society for the Study of Amphibians and Reptiles. 338 p.

Greenhouse G. 1976. Evaluation of the teratogenic effects of hydrazine, methylhydrazine, and dimethylhydrazine on embryos of *Xenopus laevis*, the South African clawed toad. *Teratology* 13:167–178.

Greer AE. 1997. Developmental and evolutionary implications of some limb abnormalities in a sample of green and golden bell frogs, *Litoria aurea* (Hylidae). *J Herpetol* 31:596–599.

Griffiths RA. 1981. Physical abnormalities and accessory limb growth in the smooth newt, *Triturus vulgaris*. *Br J Herpetol* 6:180–182.

Grillitsch B, Grillitsch H. 1989. Teratological and ontogenetic alterations to external oral structures in some anuran larvae (Amphibia: Anura: Bufonidae, Ranidae). *Fortschr Zool* 35:276–282.

Guillette Jr LJ. 1995. Endocrine disrupting environmental contaminants and developmental abnormalities in embryos. *Hum Ecol Risk Assess* 1:25–36.

Hamilton AG. 1950. Polymely in a frog. *Nature* 166:611–612.

Handyside PD. 1866. Observations on the arrested twin development of Jean Battista dos Santos, born at Faro in Portugal, in 1846. *Edinburgh Med J* 11:833–842.

Hanken J. 1983. High incidence of limb skeletal variants in a peripheral population of the red-backed salamander, *Plethodon cinereus* (Amphibia: Plethodontidae), from Nova Scotia. *Can J Zool* 61:1925–1931.

Hanken J, Dinsmore CE. 1986. Geographic variation in the limb skeleton of the red-backed salamander, *Plethodon cinereus*. *J Herpetol* 20:97–101.

Hanken J, Wassersug RJ. 1981. The visible skeleton. *Func Photogr* 16:22–26, 44.

Harfenist A, Power T, Clark KL, Peakall DB. 1989. A review and evaluation of the amphibian toxicological literature. Ottawa, Ontario, Canada: CWS. Technical Report Series No 61. 222 p.

Harmon MA, Boehm MF, Heyman RA, Mangelsdorf DJ. 1995. Activation of mammalian retinoid X receptors by the insect growth regulator methoprene. *Proc Natl Acad Sci* 92:6157–6160.

Hauptman O, Albert DM, Plowman MC, Hopfer SM, Sunderman Jr FW. 1993. Ocular malformations of *Xenopus laevis* exposed to nickel during embryogenesis. *Ann Clin Lab Sci* 23:397–406.

Hayes TB. 1997. Steroid-mimicking environmental contaminants: their potential role in amphibian declines. In: Böhme W, Bischoff W, Ziegler T, editors. Herpetologia bonnensis. Bonn, Fed Repub Ger: SEH. p 145–149.

Hays JB, Blaustein AR, Kiesecker JM, Hoffman PD, Pandelova I, Coyle D, Richardson T. 1996. Developmental responses of amphibians to solar and artificial UVB sources: a comparative study. *Photochem Photobiol* 64:449–456.

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

Hebard WB, Brunson RB. 1963. Hind limb anomalies of a western Montana population of the Pacific tree frog, *Hyla regilla* Baird and Girard. *Copeia* 1963:570–572.

Hecnar SJ. 1995. Acute and chronic toxicity of ammonium nitrate fertilizer to amphibians from southern Ontario. *Environ Toxicol Chem* 14:2131–2137.

Helgen J, McKinnell RG, Gernes MC. 1998. Investigation of malformed northern leopard frogs in Minnesota. In: Lannoo MJ, editor. Status and conservation of Midwestern amphibians. Iowa City IA: University of Iowa. p 288–297.

Hellmich W. 1929. Mehrfachbildungen von extremitäten bei amphibien (naturfunde). *Wilhelm Roux' Arch Entwicklungsmech Organ* 115:409–414.

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.

Héron-Royer. 1884. Cas tératologiques observés chez quelques têtards de batraciens anoures et de la possibilité de prolonger méthodiquement l'état larvaire chez les batraciens. *Bull Soc Zool Fr* 9:162–168.

Hillenius D. 1959. Ein zweiter fall der "Anomalie P" (Rostand) bei *Rana esculenta* Linné. *Med Klin* 54:99–100.

Hobson BM. 1958. Polymely in *Xenopus laevis*. *Nature* 181:862.

Honrubia MP, Herráez MP, Alvarez R. 1993. The carbamate insecticide ZZ-Aphox® induced structural changes of gills, liver, gall-bladder, heart, and notochord of *Rana perezi* tadpoles. *Arch Environ Contam Toxicol* 25:184–191.

Houck WJ, Henderson C. 1953. A multiple appendage anomaly in the tadpole of *Rana catesbeiana*. *Herpetologica* 9:76–77.

Howes GB. 1893. Notes on variation and development of the vertebral and limb-skeleton of the Amphibia. *Proc Zool Soc Lond* (19):268–278.

Humphrey RR. 1967. Genetic and experimental studies on a lethal trait ("short toes") in the Mexican axolotl (*Ambystoma mexicanum*). *J Exp Zool* 164:281–296.

Hvass H. 1943. Ein fall von polymelie beim wasserfrosch, *Rana esculenta* L. *Vidensk Medd Dansk Naturh Foren København* 106:233–240.

Jäckel P. 1881. Ein fünfbeiniger *Triton cristatus*. *Zool Gart* 22:156.

Johnson KJ, Scadding SR. 1991. Effects of vitamin A and other retinoids on the differentiation and morphogenesis of the integument and limbs of vertebrates. *Can J Zool* 69:263–273.

Johnson PTJ, Lunde KB, Ritchie EG, Launer AE. 1999. The effect of trematode infection on amphibian limb development and survivorship. *Science* 284:802–804.

Johnson RH. 1901. Three polymelous frogs. *Am Nat* 35:25–31.

Joly J. 1966. Polydactylie bilatérale naturelle chez des larves de *Salamandra salamandra* (L.). *Bull Soc Zool Fr* 91:336.

Kinghorn JR. 1924. Reptiles and batrachians from south and south-west Australia. *Rec Aust Mus* 14:163–183.

Kingsley JS. 1880. An abnormal foot in *Amblystoma*. *Am Nat* 14:594.

Kingsley JS. 1881. A case of polymely in the Batrachia. *Proc Bost Soc Nat Hist* 21:169–175.

Klausewitz W. 1952. Doppelseitige polydactylie bei einem männchen von *Rana esculenta*. *Senckenbergiana* 33:247–251.

Koskela P. 1974. Combination of partial adactylism and syndactylism in *Rana temporaria* L. *Aquil Ser Zool* 15:37–38.

Kuzmin SL, Dodd Jr CK, Pikulik MM, editors. 1995. Amphibian populations in the Commonwealth of Independent States: current status and declines. Moscow, Russ Fed: Pensoft. 159 p.

La Clair JJ, Bantle JA, Dumont J. 1998. Photoproducts and metabolites of a common insect growth regulator produce developmental deformities in *Xenopus*. *Environ Sci Technol* 32:1453–1461.

Lambiris AJL. 1982. Syndactyly in *Bufo maculatus* Hallowell (Amphibia: Anura: Bufonidae). *S Afr J Zool* 17:122–125.

Landois H. 1884. Ein sechsbeiniger Molch. *Zool Gart* 25:94.

Lannoo MJ, editor. 1998. Status and conservation of Midwestern amphibians. Iowa City IA: University of Iowa. 507 p.

Lauthier M. 1971. Étude descriptive d'anomalies spontanées des membres postérieurs chez *Pleurodeles waltlii* Michah. *Ann Embryol Morphog* 4:65–78.

Lazell J. 1995. *Plethodon albagula* (western slimy salamander): foot anomalies. *Herpetol Rev* 26:198.

LeBlanc GA, Bain LJ. 1997. Chronic toxicity of environmental contaminants: sentinels and biomarkers. *Environ Health Perspect* 105(Suppl 1):65–80.

Leibovitz HE, Culley Jr DD, Geaghan JP. 1982. Effects of vitamin C and sodium benzoate on survival, growth and skeletal deformities of intensively cultured bullfrog larvae *Rana catesbeiana* reared at two pH levels. *J World Maricult Soc* 13:322–328.

Levy BM. 1959. New method for the rapid determination of lathyrogenic agents. *Science* 129:720.

Liceti F. 1634. De monstrorum caussis, natura, et differentiis libri duo. 2nd edition. Patavii [Padova], Italy: Apud Paulum Frambottum. 262 p.

Lopez TJ, Maxson LR. 1990. *Rana catesbeiana* (bullfrog): polymely. *Herpetol Rev* 21:90.

Lowcock LA, Sharbel TF, Bonin J, Ouellet M, Rodrigue J, DesGranges J-L. 1997. Flow cytometric assay for in vivo genotoxic effects of pesticides in green frogs (*Rana clamitans*). *Aquat Toxicol* 38:241–255.

Loyez M. 1897. Sur un têtard de *Rana temporaria* bicéphale. *Bull Soc Zool Fr* 22:146–148.

Lüdicke M. 1971. Ein fünfbeiniger grasfrosch (*Rana temporaria* L.). *Zool Anz* 186:188–197.

Luis R, Báez M. 1987. Anomalías morfológicas en los anfibios de las Islas Canarias (Amphibia, Anura). *Vieraea* 17:295–296.

Lunel G. 1868. Sur deux cas de polymélie (membres surnuméraires) observés chez la *Rana viridis seu esculenta* (Lin.). *Mém Soc Phys Hist Nat Genève* 19:305–312.

Lynch JD. 1965. Apparent syndactyly in a Mexican frog. *J Ohio Herpetol Soc* 5:57.

Maden M. 1982. Supernumerary limbs in amphibians. *Am Zool* 22:131–142.

Maden M. 1983a. The effect of vitamin A on limb regeneration in *Rana temporaria*. *Dev Biol* 98:409–416.

Maden M. 1983b. The effect of vitamin A on the regenerating axolotl limb. *J Embryol Exp Morphol* 77:273–295.

Maden M. 1993. The homeotic transformation of tails into limbs in *Rana temporaria* by retinoids. *Dev Biol* 159:379–391.

Maden M. 1996. Retinoic acid in development and regeneration. *J Biosci* 21:299–312.

Maden M. 1997. Retinoic acid and its receptors in limb regeneration. *Cell Dev Biol* 8:445–453.

Maden M, Corcoran J. 1996. Role of thyroid hormone and retinoid receptors in the homeotic transformation of tails into limbs in frogs. *Dev Genet* 19:85–93.

Mahapatra PK, Mohanty-Hejmadi P. 1994. Vitamin A-mediated homeotic transformation of tail to limbs, limb suppression and abnormal tail regeneration in the Indian jumping frog *Polypedates maculatus*. *Dev Growth Differ* 36:307–317.

Mahendra BC. 1936. A case of polymely in the Indian bull-frog, *Rana tigrina* Daud. *Proc Indian Acad Sci* (B) 4:483–493.

Malkmus R. 1981. Bemerkungen zu einer *Triturus boscai* - Population in einem brunnenbecken der Serra de Sintra. *Bol Soc Port Ciênc Nat* 20:25–40.

Marshall GA, Amborski RL, Culley Jr DD. 1980. Calcium and pH requirements in the culture of bullfrog (*Rana catesbeiana*) larvae. *Proc World Maricult Soc* 11:445–453.

Martínez I, Álvarez R, Herráez I, Herráez P. 1992. Skeletal malformations in hatchery reared *Rana perezi* tadpoles. *Anat Rec* 233:314–320.

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

Marvin GA. 1995. *Plethodon glutinosus* (slimy salamander): morphology. *Herpetol Rev* 26:30.

Marvin GA, Hutchison VH. 1997. *Plethodon kentucki* (Cumberland Plateau woodland salamander): morphology. *Herpetol Rev* 28:199.

Matsui M, Wu G-F, Song M-T. 1993. Morphometric comparisons of *Rana chensinensis* from Shaanxi with three Japanese brown frogs (genus *Rana*). *Jap J Herpetol* 15:29–36.

Mazza F. 1888. Caso di melomelia anteriore in una *Rana esculenta* Linn. *Atti Soc Ital Sci Nat* 31:145–150.

Merrell DJ. 1969. Natural selection in a leopard frog population. *J Minn Acad Sci* 35:86–89.

Meyer-Rochow VB. 1989. A report of webbed feet in a mature female axolotl (*Siredon mexicanum*) and remarks on webbed feet in Urodela, generally. *Amphib Rept* 10:89–92.

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

Mijares-Urrutia A, Arends RA. 1999. A new *Mannophryne* (Anura: Dendrobatidae) from western Venezuela, with comments on the generic allocation of *Colostethus larandinus*. *Herpetologica* 55:106–114.

Miller CE. 1968. Frogs with five legs. *Carolina Tips* 31(1):1.

Miller DM, Tarpley J. 1996. An automated double staining procedure for bone and cartilage. *Biotech Histochem* 71:79–83.

Minucci S, Saint-Jeannet J-P, Toyama R, Scita G, DeLuca LM, Taira M, Levin AA, Ozato K, Dawid IB. 1996. Retinoid X receptor-selective ligands produce malformations in *Xenopus* embryos. *Proc Natl Acad Sci* 93:1803–1807.

Mitashov VI. 1997. Retinal regeneration in amphibians. *Int J Dev Biol* 41:893–905.

Mizgireuv IV, Flax NL, Borkin LJ, Khudoley VV. 1984. Dysplastic lesions and abnormalities in amphibians associated with environmental conditions. *Neoplasma* 31:175–181.

Mohanty-Hejmadi P, Dutta SK, Mahapatra P. 1992. Limbs generated at site of tail amputation in marbled balloon frog after vitamin A treatment. *Nature* 355:352–353.

Murphy TD. 1965. High incidence of two parasitic infestations and two morphological abnormalities in a population of the frog, *Rana palustris* Le Conte. *Am Midl Nat* 74:233–239.

Muto Y. 1969a. Anomalies in the hindlimb skeletons of the toad larvae reared at a high temperature. *Cong Anom* 9:61–73.

Muto Y. 1969b. Hindlimb development and malformations of toes in the larvae reared at high temperature in the toad, *Bufo vulgaris formosus*. *Cong Anom* 9:1–12.

Muto Y. 1970. Digital malformations in the forelimbs of the toad larvae reared at a high temperature. *Cong Anom* 10:135–147.

Muto Y. 1971. Skeletal anomalies in the feet of toad larvae reared in aerated water at a high temperature. *Cong Anom* 11:159–169.

Newman Jr SM, Dumont JN. 1983. Thiosemicarbazide-induced osteolathyrism in metamorphosing *Xenopus laevis*. *J Exp Zool* 225:411–421.

Niazi IA. 1996. Background to work on retinoids and amphibian limb regeneration: studies on anuran tadpoles—a retrospect. *J Biosci* 21:273–297.

Niazi IA, Saxena S. 1978. Abnormal hind limb regeneration in tadpoles of the toad, *Bufo andersoni*, exposed to excess vitamin A. *Folia Biol* 26:3–8.

Nishimura K. 1967. Abnormal formation of visual organs of amphibian larvae induced by radioactive rainwater. *Mie Med J* 16:263–267.

Nurminen T. 1995. Maternal pesticide exposure and pregnancy outcome. *J Occup Environ Med* 37:935–940.

O'Donoghue CH. 1910. Instances of polymely in two frogs. Together with notes on the absence of a right pre-caval vein in two frogs. *Zool Anz* 35:759–767.

Ouellet M, Bonin J, Rodrigue J, DesGranges J-L, Lair S. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. *J Wildl Dis* 33:95–104.

Ovaska K. 1997. Vulnerability of amphibians in Canada to global warming and increased solar ultraviolet radiation. In: Green DM, editor. Amphibians in decline: Canadian studies of a global problem. Volume 1, Herpetological conservation. Saint Louis MO: Society for the Study of Amphibians and Reptiles. p 206–225.

Pacces Zaffaroni N, Arias E, Lombardi S, Zavanella T. 1996. Natural variation in the appendicular skeleton of *Triturus carnifex* (Amphibia: Salamandridae). *J Morphol* 230:167–175.

Pacces Zaffaroni N, Arias E, Zavanella T. 1992. Natural variation in the limb skeletal pattern of the crested newt, *Triturus carnifex* (Amphibia: Salamandridae). *J Morphol* 213:265–273.

Paris P. 1912. Curieux cas de tératologie chez une grenouille. *C R Assoc Fr Avanc Sci* 41:449.

Pasquarelli P, Mendes EG, Costacurta L. 1981. Natural polymely in the toad *Bufo ictericus ictericus* Spix, 1824 (Amphibia-Anura). *Zool Anz* 206:292–296.

Pearson PG. 1960. A description of a six-legged bullfrog, *Rana catesbeiana*. *Copeia* 1960:50–51.

Pelgen JL. 1951. A *Rana catesbeiana* with six functional legs. *Herpetologica* 7:138–139.

Pendlebury GB. 1973. Distribution of the dusky salamander *Desmognathus fuscus fuscus* (Caudata: Plethodontidae) in Quebec, with special reference to a population from St. Hilaire. *Can Field-Nat* 87:131–136.

Politzer G. 1926. Die doppelbildungen der urodelen. II. Mitteilung. *Wilhelm Roux' Arch Entwicklungsmech Organ* 108:417–462.

Ponse K. 1941. Sur l'ectromélie bithoracique et diverses anomalies présentées par les descendants issus d'une ponte biddérienne d'un crapaud femelle. *Rev Suisse Zool* 48:545–551.

Przibram H. 1921. Die bruch-dreifachbildung im tierreiche. *Arch Entwicklungsmech Organ* 48:205–444.

Puşcariu F, Papillian VV, Gabor M, Deac C. 1973. Toxicité et effets morphopathologiques des aflatoxines chez les têtards. *Arch Roum Pathol Exp Microbiol* 32:255–267.

Rahmann H, Engels W, Grüter HJ. 1962. Doppelmißbildung einer vorderextremität bei *Rana temporaria* L. *Zool Anz* 169:449–454.

Read JL, Tyler MJ. 1990. The nature and incidence of post-axial, skeletal abnormalities in the frog *Neobatrachus centrˈalis* Parker at Olympic Dam, south Australia. *Trans R Soc S Aust* 114:213–217.

Read JL, Tyler MJ. 1994. Natural levels of abnormalities in the trilling frog (*Neobatrachus centralis*) at the Olympic Dam mine. *Bull Environ Contam Toxicol* 53:25–31.

Reichenow E. 1908. Beispiele von abweichungen in der zahl der hintergliedmaßen bei *Rana esculenta*. *Zool Anz* 32:677–682.

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

Richardson LR, Barwick RE. 1957. Faulty eruption of the fore-limb in *Hyla aurea*. *Trans R Soc New Zealand* 84:941–942.

Riggin GW, Schultz TW. 1986. Teratogenic effects of benzoyl hydrazine on frog embryos. *Trans Am Microsc Soc* 105:197–210.

Roberts JM, Verrell PA. 1984. Physical abnormalities of the limbs of smooth newts (*Triturus vulgaris*). *Br J Herpetol* 6:416–418.

Rollo CD. 1995. Phenotypes: their epigenetics, ecology and evolution. London, UK: Chapman & Hall. 463 p.

Rosine WN. 1955. Polydactylism in a second species of Amphibia in Muskee Lake, Colorado. *Copeia* 1955:136.

Rostand J. 1947. Gynogenèse et polydactylie chez le crapaud ordinaire. *C R Soc Biol* 141:629–630.

Rostand J. 1949a. Polymélie chez le crapaud ordinaire (*Bufo bufo*). *C R Soc Biol* 143:666–668.

Rostand J. 1949b. Sur diverses anomalies relevées dans une population de crapauds (*Bufo bufo*). *C R Soc Biol* 143:758–760.

Rostand J. 1950. Polydactylie chez la grenouille rousse (*Rana temporaria*) et clinodactylie chez la grenouille verte (*Rana esculenta*). *C R Soc Biol* 144:19–20.

Rostand J. 1951a. Sur la polydactylie des batraciens anoures. *Bull Biol Fr Belg* 85:113–136.

Rostand J. 1951b. Surmaturité des oeufs et anomalies chez *Rana temporaria*. *C R Soc Biol* 145:624–625.

Rostand J. 1952a. Ectrodactylie et syndactylie chez *Rana temporaria*. *C R Soc Biol* 146:4–5.

Rostand J. 1952b. Régénération de membres normaux chez les larves de *Rana esculenta* présentant l'anomalie P. *C R Soc Biol* 146:1530–1531.

Rostand J. 1955a. Effets tératogènes des rayons ultraviolets sur les larves de grenouilles. *C R Soc Biol* 149:905–907.

Rostand J. 1955b. Nouvelles observations sur l'anomalie P chez la grenouille verte (*Rana esculenta* L.). *C R Soc Biol* 149:2075–2077.

Rostand J. 1955c. Polydactylie associée à la polymélie chez *Alytes obstetricans* Laur. *C R Soc Biol* 149:1916–1918.

Rostand J. 1956a. Polymorphisme de l'anomalie E chez *Rana temporaria* L. *C R Acad Sci* 243:973–974.

Rostand J. 1956b. Sur une nouvelle anomalie (E) de *Rana temporaria* L: ectrodactylie, torsion de la queue et pupille claire. *C R Acad Sci* 242:3000–3002.

Rostand J. 1958. Les anomalies des amphibiens anoures. Paris, France: SEDES. 100 p.

Rostand J. 1959. L'anomalie P chez la grenouille verte (*Rana esculenta* L.). *Bull Biol Fr Belg* 93:7–15.

Rostand J. 1971. Les étangs à monstres: histoire d'une recherche (1947–1970). Paris, France: Stock. 85 p.

Rostand J, Darré P. 1967. Sur les conditions d'apparition de l'anomalie P chez *Rana esculenta*. *C R Acad Sci* 265:761–762.

Rostand J, Darré P. 1968. Conditions déterminantes de l'anomalie P chez *Rana esculenta*. *C R Soc Biol* 162:1682–1683.

Rostand J, Darré P. 1969. Action tératogène des déjections de certains poissons sur les larves de *Rana esculenta*. *C R Soc Biol* 163:2033–2034.

Rostand J, Jacquot M, Darré P. 1967. Nouvelles expériences sur les causes de l'anomalie P chez *Rana esculenta*. *C R Acad Sci* 264:2395–2397.

Roth M. 1978. Experimental skeletal teratogenesis in the frog tadpole. *Anat Anz* 143:296–300.

Roth M. 1988. The 2-stage neuroskeletal pathomechanism of developmental deformities of the limb skeleton. *Anat Anz* 167:271–279.

Row RWH. 1916. A frog with symmetrically abnormal hind feet. *Proc Zool Soc Lond* 87–89.

Rowe CL, Kinney OM, Congdon JD. 1998. Oral deformities in tadpoles of the bullfrog (*Rana catesbeiana*) caused by conditions in a polluted habitat. *Copeia* 1998:244–246.

Rowe CL, Kinney OM, Fiori AP, Congdon JD. 1996. Oral deformities in tadpoles (*Rana catesbeiana*) associated with coal ash deposition: effects on grazing ability and growth. *Freshwat Biol* 36:723–730.

Ruth FS. 1961. Seven-legged bullfrog, *Rana catesbeiana*. *Turtox News* 39:232.

Ryder JA. 1878. A monstrous frog. *Am Nat* 12:751–752.

Samarasinghe E. 1951. Report on the anatomical variations observed in a polymelous specimen of *Bufo melanostictus*. *Ceylon J Sci* (B) 24:181–186.

Sanchiz FB, Pérez PJ. 1974. Frecuencia de anomalías óseas en la población de *Discoglossus pictus* (Anura, Discoglossidae) de Campos (Asturias). *Bol Est Cent Ecol* 3(6):69–77.

Scadding SR. 1981. Limb regeneration in adult Amphibia. *Can J Zool* 59:34–46.

Scadding SR, Maden M. 1986a. Comparison of the effects of vitamin A on limb development and regeneration in *Xenopus laevis* tadpoles. *J Embryol Exp Morphol* 91:35–53.

Scadding SR, Maden M. 1986b. The effects of local application of retinoic acid on limb development and regeneration in tadpoles of *Xenopus laevis*. *J Embryol Exp Morphol* 91:55–63.

Schmidt KP. 1953. A check list of North American amphibians and reptiles. 6th edition. Chicago IL: University of Chicago Press and ASIH. 280 p.

Schotté OE, Harland M. 1943. Amputation level and regeneration in limbs of late *Rana clamitans* tadpoles. *J Morphol* 73:329–363.

Schultz TW, Dumont JN, Epler RG. 1985. The embryotoxic and osteolathyrogenic effects of semicarbazide. *Toxicology* 36:183–198.

Schüßler H. 1925. Larve von *Rana esculenta* L. mit drei hintergliedmaßen. *Zool Anz* 64:137–138.

Schwartz DA, LoGerfo JP. 1988. Congenital limb reduction defects in the agricultural setting. *Am J Public Health* 78:654–658.

Sealander JA. 1944. A teratological specimen of the tiger salamander. *Copeia* 1944:63.

Semlitsch RD, Moran GB, Shoemaker CA. 1981. *Ambystoma talpoideum* (mole salamander): morphology. *Herpetol Rev* 12:69.

Sessions SK, Franssen RA, Horner VL. 1999. Morphological clues from multilegged frogs: are retinoids to blame? *Science* 284:800–802.

Sessions SK, Ruth SB. 1990. Explanation for naturally occurring supernumerary limbs in amphibians. *J Exp Zool* 254:38–47.

Shubin N, Wake DB, Crawford AJ. 1995. Morphological variation in the limbs of *Taricha granulosa* (Caudata: Salamandridae): evolutionary and phylogenetic implications. *Evolution* 49:874–884.

Smith DD, Powell R. 1983. *Acris crepitans blanchardi* (Blanchard's cricket frog): anomalies. *Herpetol Rev* 14:118–119.

Sordelli F. 1876. Descrizione di una rana polimelica del Museo Civico di Milano. *Atti Soc Ital Sci Nat* 19:392–398.

Strobel P. 1876. Ulteriori cenni sulla polimelia nelle rane. *Atti Soc Ital Sci Nat* 19:385–391.

Surlève-Bazeille J-E, Cambar R. 1969. Nouvelles recherches sur les modalités de transmission de l'anomalie P chez la grenouille verte (*Rana esculenta* L.). *C R Soc Biol* 163:2034–2037.

Surlève-Bazeille J-E, Cambar R, Calas A. 1969. Premières observations histologiques sur les membres des larves de grenouille verte (*Rana esculenta* L.) atteints de l'anomalie P. *C R Acad Sci* 269:2240–2242.

Surlève-Bazeille J-E, Cambar R, Calas A. 1970. Observations sur l'infrastructure des membres de larves de grenouille verte (*Rana esculenta* L.) atteintes de l'anomalie P. *C R Acad Sci* 270:193–195.

Surlève-Bazeille J-E, Cambar R, Mauget R. 1969. Essai de transmission de l'anomalie P à diverses espèces d'amphibiens. Premiers résultats obtenus sur *Rana temporaria*. *Actes Soc Linn Bordeaux* 106(A)(2):1–7.

Takeishi M. 1996. On the frog, *Rana ornativentris*, with supernumerary limbs found at Yamada Greenery Area in Kitakyushu City, Fukuoka prefecture, Japan. *Bull Kitakyushu Mus Nat Hist* 15:119–131. [In Japanese, English abstract].

Tanaka-Ueno T, Matsui M, Wu G-F, Fei L, Takenaka O. 1999. Identity of *Rana chensinensis* from other brown frogs as assessed by mitochondrial cytochrome *b* sequences. *Copeia* 1999:187–190.

Taruffi C. 1880. Nota storica sulla polimelia delle rane. *Atti Soc Ital Sci Nat* 23:112–122.

Thornton CS, Shields TW. 1945. Five cases of atypical regeneration in the adult frog. *Copeia* 1945:40–42.

Tornier G. 1897a. Über experimentell erzeugte dreischwänzige eidechsen und doppelgliedmaßen von molchen. *Zool Anz* 20:356–361.

Tornier G. 1897b. Über operationsmethoden, welche sicher hyperdactylie erzeugen, mit bemerkungen über hyperdactylie und hyperpedie. *Zool Anz* 20:362–365.

Tornier G. 1898. Ein fall von polymelie beim frosch mit nachweis der entstehungsursachen. *Zool Anz* 21:372–379.

Tornier G. 1905. An knoblauchskröten experimentell entstandene überzählige hintergliedmaßen. *Arch Entwicklungsmech Organ* 20:76–124.

Tuckerman F. 1886. Supernumerary leg in a male frog (*Rana palustris*). *J Anat Physiol* 20:516–519.

Tyler MJ. 1976. Frogs. Sydney, Australia: Collins. 256 p.

Tyler MJ. 1989. Australian frogs. Ringwood (Victoria), Australia: Viking O'Neil. 220 p.

Tyler MJ, Davies M, Walker KF. 1985. Abrasion injuries in burrowing frogs (Amphibia, Anura) from the Northern Territory, Australia. *Zool Anz* 214:54–60.

Uehlinger V. 1969. Une mutation récessive (pd) déterminant la polydactylie chez *Xenopus laevis* D. (Batraciens anoures). *J Embryol Exp Morphol* 21:207–218.

Vallisneri A. 1733a. Opere fisico-mediche. Volume 1. Venezia [Venice], Italy. 469 p.

Vallisneri A. 1733b. Opere fisico-mediche. Volume 2. Venezia [Venice], Italy. 551 p.

Vallisneri A. 1733c. Opere fisico-mediche. Volume 3. Venezia [Venice], Italy. 676 p.

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

Veith M, Viertel B. 1993. Veränderungen an den extremitäten von larven und jungtieren der erdkröte (*Bufo bufo*): analyse möglicher ursachen. *Salamandra* 29:184–199.

Vershinin VL. 1989. Morphological anomalies in urban amphibians. *Ékologiya* (3):58–66. [In Russian].

Vershinin VL. 1995a. Status of amphibian populations in anthropogenic landscapes of the Ural, Siberia and the Far East. In: Kuzmin SL, Dodd Jr CK, Pikulik MM, editors. Amphibian populations in the Commonwealth of Independent States: current status and declines. Moscow, Russ Fed: Pensoft. p 88–90.

Vershinin VL. 1995b. Types of morphological anomalies of amphibians in urban regions. In: Kuzmin SL, Dodd Jr CK, Pikulik MM, editors. Amphibian populations in the Commonwealth of Independent States: current status and declines. Moscow, Russ Fed: Pensoft. p 91–98.

Virey J-J. 1819. Observation et description de deux individus sexdigitaires. *J Complém Dict Sci Méd* 4:327–330.

Vizotto LD, Taddei VA, Cais A. 1977. Pseudomelia em *Phrynohyas hebes* (Cope, 1862) (Anura-Hylidae). *Naturalia* 3:35–43.

Volpe EP. 1970. Understanding evolution. 2nd edition. Dubuque IA: Brown. 175 p.

Wagner G. 1913. On a peculiar monstrosity in a frog. *Biol Bull* 25:313–317.

Washburn FL. 1899. A peculiar toad. *Am Nat* 33:139–141.

Wassersug RJ. 1976. A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technol* 51:131–134.

Winslow GM. 1904. Three cases of abnormality in urodeles. *Tufts Coll Stud* 1(8):387–410.

Witschi E. 1952. Overripeness of the egg as a cause of twinning and teratogenesis: a review. *Cancer Res* 12:763–786.

Witschi E, Chang CY. 1954. Hereditary polymelia combined with edema and sex reversal in the toad. *Anat Rec* 118:370.

Woitkewitsch (Voitkevich) AA. 1955. Magnification of limb bud in frogs. *Priroda* (12):100–102. [In Russian].

Woitkewitsch AA. 1959. Natürliche mehrfachbildungen an froschextremitäten. Jena, Fed Repub Ger: VEB G Fischer Verlag. 82 p.

Woitkewitsch (Woïtkewitch) AA. 1961. Le développement des extrémités surnuméraires chez les amphibiens. *Bull Biol Fr Belg* 95:569–600.

Woitkewitsch (Voitkevich) AA. 1963. Symmetrical disorders of the blastemata of frog hind legs. *Dokl Akad Nauk SSSR* 152:1479–1482. [In Russian].

Woitkewitsch (Voitkevich) AA. 1965. Mass formation of additional hind extremities in the lake frog. *Zh Obshch Biol* 26:56–62. [In Russian, English abstract].

Woodland W. 1908. A curious instance of polymely in the common frog. *Zool Anz* 32:354–357.

Worrest RC, Kimeldorf DJ. 1975. Photoreactivation of potentially lethal, UV-induced damage to boreal toad (*Bufo boreas boreas*) tadpoles. *Life Sci* 17:1545–1550.

Worrest RC, Kimeldorf DJ. 1976. Distortions in amphibian development induced by ultraviolet-B enhancement (290–315 nm) of a simulated solar spectrum. *Photochem Photobiol* 24:377–382.

Worthington RD. 1974. High incidence of anomalies in a natural population of spotted salamanders, *Ambystoma maculatum*. *Herpetologica* 30:216–220.

Wright KM. 1996. Amphibian husbandry and medicine. In: Mader DR, editor. Reptile medicine and surgery. Philadelphia PA: WB Saunders. p 436–459.

Wu HW, Liu CK. 1941. A case of polymely in the frog, *Rana nigromaculata*. *Sinensia* 12:75–80.

Zavanella T, Pacces Zaffaroni N, Arias E. 1984. Abnormal limb regeneration in adult newts exposed to the fungicide maneb 80. A histological study. *J Toxicol Environ Health* 13:735–745.

Zile MH. 1992. Vitamin A homeostasis endangered by environmental pollutants. *Proc Soc Exp Biol Med* 201:141–153.