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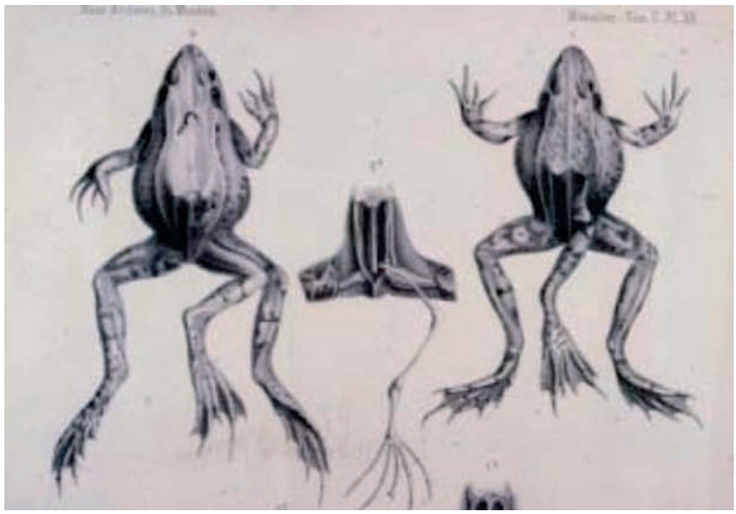

Fig. 1

The Case of the Deviant Toad:

An introduction to the proximate causes for limb deformities in amphibians

Brandon Ballengée & Stanley K. Sessions 2010

Accounts of wild-caught frogs with extra (or supernumerary) limbs first appear in 18th and 19th century medical and natural history literature (Fig. 1). Sometimes referred to as "monsters", these specimens were usually reported as individual anomalies with only one or a few individuals found at given sites. By the middle 20th century some published reports included high frequencies of amphibians with deformed limbs among wild populations in Asia and Europe.[1] All organisms show low frequencies of developmental deformities, and in amphibians the frequency usually ranges between 1% and 5% among wild populations, but can be as high as 70% or more in cohorts of newly metamorphosed frogs.[2]

Fig. 2

The occurrence of limb abnormalities in wild populations of frogs first received significant international media attention in 1990 when Sessions and Ruth reported their findings of thousands of frogs and salamanders with supernumerary limbs and other deformities from some ponds in northern California.[3] The animals had striking abnormalities; some with extra hind limbs while a few were missing limbs. Visually they recalled malformed human children: victims of Agent Orange, thalidomide, or the radioactive aftermath of Chernobyl. However, Sessions and Ruth discovered that these deformities were caused by a naturally occurring parasitic flatworm (trematode). This discovery was reported in major newspapers around the world, but was not yet considered an environmental problem.

Five years after the publication of Sessions and Ruth (1990), a group of Minnesota school children found some similarly deformed frogs while on a field trip.[4] Alarmed, their teacher contacted the Minnesota Pollution Control Agency who helped the class develop a web page to report their findings. Media coverage of the Minnesota Case strongly implied that the deformities were caused by chemical pollution. Soon thereafter, North American citizens were asked to report such frogs to the United States Geological Survey's NARCAM (North American Reporting Center for Amphibian Malformations). Within a few years, numerous reports of deformed amphibians came from all over the continent. Today, « outbreaks » of limb abnormalities among wild populations (often involving minor abnormalities, such as missing digits, and one or two individuals) have been reported from every continent except Antarctica. This has remained one of most prominent environmental issues of the last 15 years.[5]

By now we know that the vast majority of both recent and historic reported abnormalities fall into more-or-less two distinct categories, involving mainly the hind limbs: either extra hind limbs and various associated deformities, or missing limbs or limb parts, also with various associated deformities (Fig. 2). Both categories of deformities have been reported in urodeles (salamanders and newts) as well as anurans (frogs and toads), but mainly only those species that have aquatic larvae (an important clue as to what is going on, please see Sessions and Ballengée in this publication). A number of hypotheses have been put forth to explain these two categories, including chemical pollution, UV-B radiation, parasites and predation as well as potential combinations.[6]

Some researchers suggest that what we are finding in the wild frogs are actually intrinsic malformations (like birth defects) caused directly by chemical pollutant(s) or UV-B radiation.[7] Alternatively, our research has built substantial evidence suggesting that permanent deformities in young frogs among wild populations stem from injuries incurred while still tadpoles caused by parasites and predators – even by cannibalism by other tadpoles! (for review see Sessions and Ballengée, in this publication).

Already by 1990, the senior author of this text and Ruth had determined that a parasitic trematode could account for frogs with extra limbs. This parasitic flatworm was shown to target and attack developing hindlimbs in tadpoles. Once embedded, the parasite forms a cyst inside the host tadpole body disrupting normal limb development. This results in the growth of additional limbs and limb parts through cellular intercalation (a powerful mechanism of pattern formation involving

Fig. 3

the disruption of the spatial relationships of signaling cells in developing limbs, Please see Sessions and Ballengée, this publication). Over 40 papers by the senior author and colleagues have continued to demonstrate and prove the trematode hypothesis beyond a reasonable doubt.[8] The fact is that trematodes can and do cause frogs with extra legs. Of course, important questions still remain unanswered, including: are mass trematode infections emblematic of new disease in amphibians? And, do secondary factors, such as changes to wetland habitats and other environmental stressors, substantially increase amphibian susceptibility to trematode infection? There is still research to be done.

Solving the mystery of extra limbs was a significant achievement, but it left the largest category of deformed frogs (those with missing limbs) unsolved. Although not as dramatic looking as frogs with multiple extra limbs, these turned out to be much more difficult to explain. Collectively, for more than ten years we have surveyed wild populations of frogs throughout North America as well as sampled specimens in Asia, Australia and Europe finding missing limb deformities among almost every population. The frogs themselves contained important clues: Firstly, the majority of the collected frogs appeared healthy except for the affected limbs, we would suspect the opposite if contamination by pollution was directly altering fitness. Next, the deformities occur mostly in hind limbs and we would expect the opposite if chemicals were inducing them as anuran forelimbs develop in the gill chamber where they would be exposed to a constant stream of polluted water. In our field work, we have found young frogs and tadpoles with injured limbs along with deformed individuals, suggesting a correlation.

In 2006, on a pilot survey in Yorkshire, England, numerous deformed Toadlets were found with abnormalities at the Havercroft Village Green Pond outside of Wakefield. The metamorphic toads showed an array of deformities ranging from partial legs, truncated limbs, to both hind limbs fully removed. The deformed toads, at least superficially, resembled prior specimens collected in our North American studies. The next summer a series of experiments were conducted to ascertain the effect of potential pollutants in the Havercroft site water and sediment on normal tadpoles – all yield negative results. By 2008, our focus shifted to carefully controlled experimental simulations with toad tadpoles at different stages of development with the varied aquatic predators. These included fish, other amphibians and several species of arthropods, including dragonflies at the larval nymph stage. We observed three species of Dragonfly nymphs capturing and injuring tadpoles. Of these *Sympetrum sp.* (probably either *S. striolatum* or *S. sanguineum*) were particularly effective at inducing non-lethal injuries to developing hind limbs creating permanent deformities identical to those found in the wild (Fig. 3, from Ballengée and Sessions, 2009).

These studies resulted in the first publication of strong experimental and field evidence supporting the idea that missing limbs in deformed amphibians can be caused by a specific predator: dragonfly nymphs.[9] These insect larvae (and certain other predators with mouth parts that are too small to consume an entire tadpole) practice selective predation, attacking or capturing tadpoles and gnawing off their protruding hind limbs, often causing permanent limb deformities in frogs that survive to metamorphosis. This coupled with the well-

known decline in regenerative ability at onset of later stages of tadpole development readily accounts for the idiosyncratic range of deformities found in wild populations. As with any study, there are still future questions to address but after more than a decade and half worth of research, a consensus is now beginning to emerge that the proximate causes for anuran limb deformities are parasites and predators. Those featuring extra limbs are most likely caused by a parasite, specifically the trematode *Ribeiroia ondatrae* and deformities featuring missing limbs or limb segments are most likely caused by selective predation by tadpole predators such as larval dragonfly nymphs.

List of Figures:

Figure 1. Multi-limbed frog from Duméril, A. 1865. Observations sur la monstruosite dite polymelie ou augmentation du nombre des membres chez les batraciens anoures. *Nouv. Arch. Mus. Hist. Nat.* 1 :309-319

Figure 2. Missing limb and multi-limbed deformities in Pacific treefrogs/ *Pseudacris (Hyla) regilla*, collected in Oregon, USA (photograph by Stanley K. Sessions)

Figure 3. Deformed hind limbs in wild-caught B. bufo tadpoles (top row) compared with hind limb deformities in tadpole (bottom row) induced by selective predation by captive dragonfly nymphs from Ballengée B, Sessions SK, 2009

Footnotes

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