

# Do Predators Cause Frog Deformities? The Need for an Eco-Epidemiological Approach

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## ABSTRACT

Renewed controversy has emerged over the likely causes and consequences of deformed amphibians, particularly those with missing limbs. The results of a series of experiments by Ballengée and Sessions (2009) implicate aquatic predators (i.e. dragonfly larvae) in causing such abnormalities. Skelly and Benard (2010), however, argued that the small scale of these experiments and the absence of a correlation between predator abundance and deformity frequencies in natural amphibian populations undermine such a conclusion. Drawing upon our experiences with frog malformations, we suggest that the study of amphibian deformities has been hindered by two, interrelated problems. First, empirical studies often fail to critically define the expected baseline level of abnormalities and differentiate between "epidemic" and "endemic" frequencies of malformations. Second, recognizing the likelihood of multiple causes in driving amphibian malformations, continued research needs to embrace a "multiple lines of evidence" approach that allows for complex etiologies by integrating field surveys, diagnostic pathology, comparative modeling, and experiments across a range of ecological scales. We conclude by highlighting the results of a recent study that uses this approach to identify the role of aquatic predators (i.e., fishes and dragonflies) in causing high frequencies of deformed frogs in Oregon. By combining long-term data, comparative data and mechanistic experiments, this study provides compelling evidence that certain predators do cause deformities under ecologically relevant conditions. In light of continuing concerns about amphibian deformities and population declines, we emphasize the need to integrate ecological, epidemiological, and developmental tools in addressing such environmental enigmas. *J. Exp. Zool. (Mol. Dev. Evol.)* 314B:515–518, 2010. © 2010 Wiley-Liss, Inc.

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Over the past 15 years, scientists have debated the causes and implications of amphibian limb deformities, which first gained widespread attention in North America during the mid 1990s (e.g. Souder, 2000). Substantial progress has been made in determining which factors do (and which do not) cause limb deformities (see Ankley et al., 2004; Johnson et al., 2010). For example, we now know that trematode parasite infection (*Ribeiroia ondatrae*) is an important cause of some of the most severe malformations, including extra limbs, bony triangles, and skin webbings, and likely contributed to many observations from originally reported "hotspots" in Minnesota and the western United States (Johnson et al., '99, 2002, 2010; Blaustein and Johnson, 2003). However, parasite infection is unlikely to explain reports involving anurans with predominantly missing or partially missing limbs, which

are among the most commonly observed abnormality types (Guderyahn, 2006; Skelly et al., 2007; Reeves et al., 2008).

Renewed debate and disagreements have recently arisen over the causes of such missing-limb abnormalities. Ballengée and Sessions (2009) provided experimental evidence that larval

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dragonflies, which are common predators in freshwater ecosystems, can capture tadpoles and remove portions of the tail or developing hind limbs without killing the tadpole. The resulting amputation, combined with varying levels of regeneration following the attack, resulted in a range of post-metamorphic deformities indistinguishable from those observed in natural systems. Based on these results, the authors suggested that predation is the major cause of missing-legged frogs in nature. In a perspective piece, however, Skelly and Benard (2010) disagreed, countering that Ballengée and Sessions provided insufficient evidence to support a role for predators because the study was (i) based on small-scale laboratory experiments and (ii) failed to provide quantitative evidence for a link between predator density and abnormality frequency, thus precluding an assessment of the explanatory power of predators at realistic scales. They argued that making broad conclusions on the basis of such experiments was “premature” and “counterproductive” (Skelly and Benard, 2010).

Drawing upon our own collective experiences studying deformed frogs, including examinations of tens of thousands of frogs over the last two decades, we contend that this debate and the ongoing study of amphibian abnormalities continues to be hindered by two interrelated problems. First, many of the disagreements over the potential causes of deformed frogs stem from differences in how the “problem” is defined, which sharply influences one’s perception of causality. It is important to begin by defining what is expected. Some individuals with morphological abnormalities are found in all populations, whether human, livestock, or amphibian, owing to mutation, trauma, and errors in development. The challenge, therefore, lies in differentiating between “normal” levels of abnormalities and elevated levels. Most research supports a baseline frequency of limb abnormalities at somewhere between 2 and 5% of the population, with populations exhibiting significantly higher frequencies classified as “hotspots” (see Ouellet, 2000; Johnson et al., 2010). This distinction is important because studies attempting to explore the causes of baseline levels of abnormalities (<5%) will generate very different conclusions about the importance of potential causes relative to those investigating hotspots, where abnormality frequencies may affect >20% of emerging frogs in a population.

Epidemiologists face a similar challenge in trying to differentiate between endemic (or expected) levels of an infection and what occurs during an epidemic, when the frequency of a particular pathology becomes elevated. For example, ~3% of humans are born with birth defects ranging from congenital heart disease to spina bifida (Robinson and Linden, '93). This is “normal.” During the 1950s, however, when the drug thalidomide was prescribed to treat morning sickness, more than 10,000 babies were born with severe birth defects (e.g. Zimmer, 2010). Thalidomide was a poor explanation for base-rate levels of abnormalities, but it was a strikingly good predictor of elevated frequencies of particularly severe malformations, including phocomelia (seal limbs). In a similar way, amphibian studies

that focus on malformation hotspots may arrive at very different conclusions about potential causes from those that study wetlands with baseline levels of abnormalities. For example, a recent study in Vermont found no relationship between trematode parasite infection and the occurrence of abnormal frogs (Skelly et al., 2007). However, the overall frequency of abnormal frogs in the study was 3%, or well within the expected baseline range. Thus, the occurrence of “normal” levels of abnormalities in Vermont, combined with the rarity of *Ribeiroia*, could be used as evidence in support of the parasite hypothesis, particularly in light of the close association between *Ribeiroia* and malformation hotspots (5–90% frequency) in the western United States (Johnson et al., 2002). This is not to say that parasites explain all hotspots or that understanding the causes of below-baseline deformity levels is unimportant; rather, such examples underscore the importance of defining exactly what one is trying to explain (e.g. endemic vs. epidemic levels).

A second challenge in studying amphibian deformities centers on how we understand and investigate causality in ecological systems. As pointed out by Skelly and Benard (2010), just because something can cause abnormalities in the laboratory doesn’t mean it actually does cause them in nature. Epidemiologists continually wrestle with this problem, particularly in the face of emerging infectious diseases. Classical reductionist approaches to studying disease causation, such as Koch’s Postulates, which were developed primarily for directly transmitted bacterial infections, are often inadequate or unethical in the face of contemporary diseases, many of which are zoonotic (involve wildlife hosts), cannot be cultured in the laboratory, or involve environmental cofactors (Smithells, '63; Plowright et al., 2008). In recognition of this complexity and the inadequacy of single-factor/single-outcome explanations, epidemiologists and ecologists alike are moving toward using a “multiple lines of evidence” approach to understand causation, which typically involves the development of competing hypotheses and the combination of experiments, field surveys, long-term data, modeling, and diagnostic pathology to evaluate the relative contributions of each factor (see Plowright et al., 2008). We contend that a similar approach is needed in the study of amphibian deformities.

We recently published the results of a 20-year study that uses this approach to explain missing limb abnormalities in Oregon amphibians (Bowerman et al., 2010). One of us (J.B.) has been monitoring patterns of limb abnormalities in western toads (*Bufo boreas*) at a deformity hotspot in Oregon since 1988. These abnormalities are dominated by missing limbs or portions of a limb and can affect up to 35% of emerging toads (mean frequency between 1988 and 2008 = 12.9%,  $n = 13,443$ ). By combining comparative sampling, lab experiments, enclosure studies, and long-term data, we present compelling evidence that the abnormalities are caused by an introduced fish (the 3-spined stickleback, *Gasterosteus aculeatus*). Experiments confirmed that sublethal predatory attacks by sticklebacks could cause the same

types and frequencies of abnormalities observed in nature, whereas comparisons among wetlands with and without sticklebacks and among years with high and low stickleback abundance reinforced the link between fish attack and toad abnormalities. Further, a simple enclosure study eliminated missing limb deformities in experimental animals, even while they persisted among unprotected conspecifics outside the enclosures. Similarly, Fiedler and Corkran (personal communication) have suggested that introduced speckled dace (*Rhinichthys osculus*) were the principle cause of high levels (1–40%) of missing limb injuries in toads from a site in northern Oregon between 1997 and 2002.

But fish are not the only predators capable of causing such abnormalities. At a series of high elevation (> 2,000 m) wetlands in eastern Oregon, larval dragonfly predators (*Somatochlora albicincta*) were linked to high levels (5–20%) of abnormalities in Cascades frogs. Again, experimental studies established the ability of these predators to cause field-observed abnormalities (Bowerman et al., 2010), and comparative sampling linked the abundance of dragonfly larvae with the frequency of abnormalities (C. Pearl, unpublished). But, perhaps more importantly, we repeatedly witnessed (and photographed) dragonfly larvae that had captured tadpoles by a hind limb and eventually severed the appendage before releasing the wounded animal (Fig. 1). Wetlands with abnormalities either lacked *Ribeiroia* infection or supported too little infection to explain the observed abnormalities (Bowerman et al., 2010).

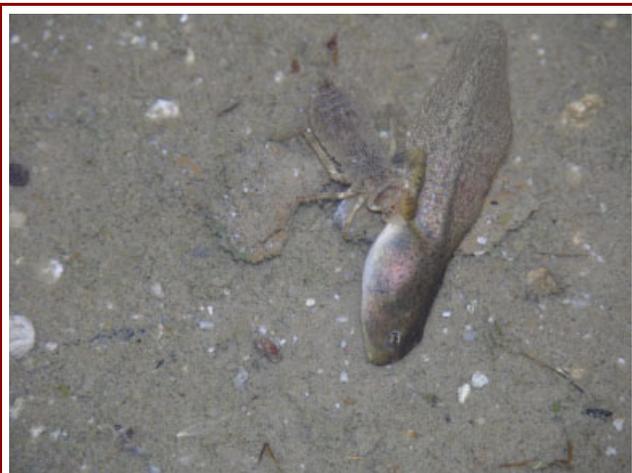
Collectively, these studies provide convincing evidence that sublethal predator attacks not only have the potential to cause missing-legged frogs in the laboratory but that they do, in fact, cause high levels of such abnormalities in some natural wetlands. These and other studies of amphibian deformities in North

America and Europe also emphasize that, despite the recent debate's focus on dragonfly larvae, missing limb abnormalities can be caused by a variety of aquatic predators, including small fishes, leeches, crabs, crayfishes, aquatic insect larvae, and other amphibians (Duellman and Trueb, '94; Van Gelder and Stribosch, '95; Bohl, '97; Johnson et al., 2001, 2006; Gray et al., 2002).

Why then, as asked by Skelly and Benard, don't we see high frequencies of missing limb abnormalities in virtually every amphibian population, given the ubiquity of aquatic predators? We echo this question, and suggest that one of the most important priorities for future research on amphibian abnormalities is determining under what circumstances each potential cause is important. This requires, however, that studies move beyond single-factor explanations and recognize the importance of environmental cofactors and context-dependent effects. With respect to predators, elevated levels of amphibian limb abnormalities could occur when the predator is extremely abundant or invasive, as was the case with sticklebacks and speckled dace, or when characteristics of the habitat increase the risk of sublethal attack. For example, among the high elevation sites in Oregon, the wetlands supported a low abundance and diversity of alternative prey and little habitat refugia, potentially increasing the likelihood of attack on tadpoles. Moreover, the short growing season causes dragonfly larvae to overwinter for one or more years, such that *Somatochlora* larvae are often large enough to attack tadpoles but too small to consume them entirely. Similar hotspots of missing-legged frogs in Alaska have been suggested to result from interactions between dragonfly predators and toxic contaminants (Reeves et al., 2010).

Today, nearly 15 years after amphibian deformities first gained national attention, it should be increasingly apparent that multiple causes contribute to the etiology of amphibian malformations. The fundamental challenge, therefore, is to understand when and under what circumstances different factors are important contributors. For example, in the last decade we have come to understand that trematode parasite infection is important in explaining high frequency accounts of severe limb malformations, particularly in the western and Midwestern United States, but that *Ribeiroia* is unlikely to account for cases involving exclusively missing limbs. The recent study by Ballengée and Sessions (2009) as well as our own (Bowerman et al., 2010) help to clearly establish that (a) sublethal predator attacks can cause missing limbs in amphibians that are similar to field observations, and (b) at least in some wetlands, such attacks likely explain the high frequency of these abnormalities. This is not to say that predators explain all or even most missing-legged frogs observed in nature, but growing evidence suggests that predatory attack cannot be summarily dismissed on the basis of indirect argument or speculation. Earlier claims that predators would not selectively remove all or part of a limb and, even if they did, the amphibian could not survive the encounter, have effectively been falsified (see Lannoo, 2008).

In keeping with the epidemiological approach to studying causation, ongoing studies of amphibian deformities need to



**Figure 1.** Dragonfly larva (*Somatochlora albicincta*) in the process of severing a hind limb from a Cascades frog (*Rana cascadae*) tadpole, which was subsequently released without the limb. Image was captured in situ at Broken Top wetlands, Oregon.

consider the likely role of multiple causative agents, including parasites, predators, and other hypothesized causes or cofactors. Fortunately, this can be a relatively straightforward process from an empirical standpoint. For example, the absence of *Ribeiroia* within necropsied frogs or of its requisite snail hosts from a given wetland strongly suggests trematode infection is not the primary cause of observed abnormalities. Similarly, in situ enclosures with variable mesh sizes can be used to selectively exclude predators, parasites, or both, affording researchers an inexpensive method to experimentally assess whether abnormalities persist in the absence of such agents, which might suggest a toxicological or genetic pathway (e.g. Kiesecker, 2002; Bowerman et al., 2010; Reeves et al., 2010). Despite the relative simplicity of such methods, few studies have explored the role of more than one factor in explaining amphibian deformities. In light of continuing concerns about the implications of amphibian deformities as well as widespread declines in native amphibian populations, we emphasize the need to incorporate a “multiple lines of evidence” approach that integrates ecological, epidemiological, and developmental tools in addressing such environmental enigmas.

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