COMMENTARY AND PERSPECTIVE

Mystery Unsolved: Missing Limbs in Deformed Amphibians

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ABSTRACT

Ballengee and Sessions (2009) claim that predatory attacks by small predators such as *Sympetrum* dragonfly larvae are sufficient to explain amphibian limb deformities in which the limb is partly or completely missing. This deformity type, the most common in nature, is not well explained by *Ribeiroia* infection which has also been nominated as a mechanism for limb deformities. We argue that the conclusions of the Ballanegee and Sessions study are not well founded. In part this is because the authors have provided no quantitative analysis of the association between limb deformities and predator densities. Our own data on frequencies of limb deformities suggest that missing hind limbs are often extremely rare even when *Sympetrum* and other small predators are common. While predatory attacks may contribute to observations of limb deformities, further study will be required to elucidate their role; other potential mechanisms deserve study as well. It is premature, and counterproductive, to draw any conclusions regarding the mechanisms behind the most common limb deformities recorded in natural populations. *J. Exp. Zool. (Mol. Dev. Evol.) 314B:179–181, 2010*. © 2009 Wiley-Liss, Inc.

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Ballengee and Sessions (2009) take on an important, lingering mystery in the environmental sciences. Following more than a decade of research (e.g., Ouellet et al., '97; Johnson et al., '99; Sessions et al., '99), amphibian limb deformities remain poorly understood; this is a woeful track record badly in need of attention. Based on their study of developmental responses to predatory attacks by dragonfly larvae Ballengee and Sessions (2009) claim to have solved the problem, sharing their belief that, "…most hind limb deformities in wild-caught anurans are the result of natural regenerative responses to traumatic injuries from selective predation and, in the case of extra limbs, parasitic infection". This resolution would be welcome if it were warranted by the data. Unfortunately, this is far from true. As a consequence, their study is likely to confuse rather than clarify efforts to gain a better understanding of the causes of limb deformities in nature.

The reasoning behind the study conclusions seems to go something like: larval dragonflies such as *Sympetrum* can remove or injure anuran hind limbs during laboratory trials such that resulting developmental abnormalities of the limbs are morphologically similar to those found in natural populations. The authors argue that they have shown that it can happen so there is no need to invoke other explanations. However, the logic employed by the authors to draw ecological inference has been obsolete for several decades. While early 20th Century ecologists believed that laboratory studies were adequate means to establish a mechanism as a generally sufficient explanation for patterns in nature (such as Gause's *Paramecium* experiments (Gause, '34)), subsequent field study and experimentation showed that the variety of mechanisms operating in nature vastly exceeds that conceived and evaluated by laboratory based scientists and demonstrated that context dependence was rife (Kingsland, '85). In short, just because something can happen does not mean that it does happen (Carpenter, '96; Skelly, 2002), and even if it is happening it does not mean that it is happening at rates meaningful for a process or pattern of interest.

In the example at hand, key data are missing. The study provides no quantitative analysis evaluating whether the composition and density of predators is related to the frequency and composition of limb deformities across a set of wetlands. There are no results from field experiments in which purported selective predators are excluded, resulting in the disappearance of limb deformities in minus predation treatments. These types of

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information will be critically important before reaching any sort of conclusions about the importance of the "selective predation" hypothesis in nature.

One of the major obstacles to the "selective predation" hypothesis is that predators such as *Sympetrum* and other dragonfly larvae are so widely distributed, much more widely distributed than sites with missing limb amphibians. Our own perspectives on this issue have been influenced by long-term data we and colleagues have been collecting at the E. S. George Reserve in Michigan (e.g., Skelly et al., '99, 2003; Werner et al., 2007a,b, 2009). Between 1996 and 2008, a set of up to 37 ponds was sampled each year as part of this effort, totalling 481 pond years of data. During more than one-third of those pond years, larval dragonflies of the genus *Sympetrum* achieved densities of >1 individual per square meter; they were among the most common dragonflies in our samples (McCauley et al., 2008). In spite of their widespread distribution and often high local density, we have encountered less than 10 individuals with hind limb deformities of any description out of 36,151 individuals examined across 8 anuran species collected. Figure 1 presents these data highlighting the high frequency of pond years for which there were no limb deformities found in spite of the frequent occurrence of high *Sympetrum* densities. While these results include larval anurans of all possible developmental stages from hatching through the onset of metamorphosis, they are reinforced by findings from a study of wood frog (*Rana sylvatica*) metamorphs in 6 drift-fenced ponds at the E. S. George Reserve. Among the 22,482 metamorphs examined during the last 4 years, just 0.07% of all wood frogs have been discovered with deformed or missing hind limbs. As in the broader set of E.S. George Reserve ponds, the drift-fenced sites frequently have high *Sympetrum* densities. Collectively, these field patterns (along with comparable results from extensive sampling in Connecticut between 1996 and the present) offer no support for the contention that hind limb deformities should be common in the presence of small predators such as *Sympetrum*. Many more such datasets should be examined before a clear sense of the natural history of associations between predators and limb deformities can be claimed.

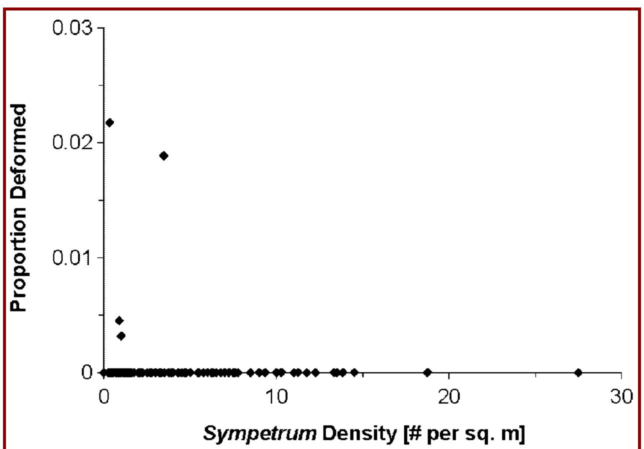

Figure 1. Hind limb deformities among 8 species of premetamorphic anurans collected 37 ponds at the E.S. George Reserve (Michigan) between 1996 and 2008. The proportion of individuals with abnormal hind limbs is plotted against the density of *Sympetrum* dragonfly larvae. *Sympetrum* density represents the aggregate number of individuals per meter squared of 5 species: *S. internum*, *S. obstrusum*, *S. rubicundulum*, *S. semicinctum* and *S. vicinum*. Details on collecting methods are given in Werner et al. (2007a).

As the authors state, scientists have long known that aquatic predators are capable of removing and injuring tadpole hind limbs (e.g., Martof, '56). Their experiments offer additional detail on this phenomenon, but they do not change the fundamental state of our ecological understanding. While predation may be important, we still do not know whether it is and further laboratory work by itself is unlikely to change the situation.

Part of our concern with the Ballengee and Sessions study is prompted by the history of research on amphibian limb deformities. For several years, study after study contended that infection by *Ribeiroia* (a trematode parasite) was the prime cause for limb deformities in nature (e.g., Johnson et al., '99; Sessions et al., '99; Johnson et al., 2001; Stopper et al., 2002; Johnson and Sutherland, 2003; Sessions, 2003). To many observers, it appeared that these studies represented a solution to the mystery of amphibian deformities. It was only after large scale field studies showed that *Ribeiroia* was absent from many locations and even regions with high frequencies of limb deformities (e.g., Lannoo et al., 2003; Taylor et al., 2005; Skelly et al., 2007) that alternatives are again being considered. This is a positive development, but the premature conclusions reached once earlier in the study of limb deformities should prompt careful consideration of alternative hypotheses. Any impression that the limb deformity mystery is solved is unwarranted and unproductive. There is much work to do before we will be able to declare that we understand the origin of most amphibian limb deformities.

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