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# INTERFERENCE COMPETITION IN TADPOLES: ARE MULTIPLE AGENTS INVOLVED?

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The inconsistent results of recent research concerning water-borne growth inhibitors in tadpoles is part of a bothersome trend that has occurred over the last thirty years. Several factors have contributed to confusion about the agents involved in growth inhibition, and the role that these agents play in regulating natural populations of tadpoles. First, there has been much disagreement on the exact nature of growth-inhibiting agents found in tadpole guts, with candidates including proteinaceous compounds, fungi, and algae (Beebee, 1991). Second, researchers have not used standardized laboratory procedures. Differences in temperature, tadpole densities, tadpole size distributions, food types, feeding schedules, and methods used to isolate and culture growth-inhibiting agents may in part explain why conflicting results have sometimes emerged over the years. Beebee (1991), for example, found that the degree of growth inhibition in *Prototheca* increased with the number of cells that tadpoles were fed, while West (1960) found the opposite. Although *Prototheca* has been implicated as the primary growth-inhibiting agent in tadpoles, Steinwascher (1978; 1979*a,b*) found that water-borne agents other than *Prototheca* inhibit tadpole growth in the laboratory. Specifically, he found that tadpole growth is inhibited in a dose-dependent manner when tadpoles are fed different concentrations of yeast (*Candida humicola*) that were isolated from a natural habitat. Finally, all but the most recent studies were conducted under artificial laboratory conditions that may not reflect the biological complexities of natural ponds.

Despite these limitations, there are many points upon which researchers do agree. One is that *Prototheca* can reach very high levels in crowded laboratory animals and can strongly inhibit tadpole growth. A second is that *Prototheca* can inhibit the growth of laboratory animals in a dose-dependent manner that could potentially act as a regulating agent in natural populations. A third is that *Prototheca* occurs in many natural populations. Finally, *Prototheca* infections are not species-specific (Licht, 1967) so that *Prototheca* could potentially be important in mediating competitive interactions in tadpole guilds that occur in breeding ponds.

My research (Petranka, 1989; Biesterfeldt, Petranka, & Sherbondy, 1993) has focused on determining whether water-borne growth inhibitors play a key role in regulating natural anuran populations. We have addressed this primarily by testing water collected from natural breeding ponds for inhibitory qualities, by conducting field experiments that control the degree to which test animals are exposed to tadpole faeces, and by examining the relationship between *Prototheca* densities in wild-caught tadpoles and the extent to which water from natural breeding sites is inhibitory. Surprisingly, we found that in most cases water from ponds containing high densities of tadpoles was not inhibitory. Although *Prototheca* appears to be present in most natural populations of wood frog tadpoles in North Carolina, we find it to be uncommon or rare in faecal samples from individual tadpoles, and have never found it to occur at the high levels that typify laboratory stock. Previous laboratory studies have shown that *Prototheca* will not stunt the growth of laboratory stock when at low densities (Richards, 1962). Consequently, we have questioned whether levels of *Prototheca* infections in wild-caught tadpoles are sufficient to cause growth inhibition.

At wood frog sites where growth inhibitors were detected and *Prototheca* was present, was *Prototheca* acting as a density-dependent regulating agent? Although this question cannot be answered with certainty, we did not find compelling evidence to support this hypothesis. First, *Prototheca* was relatively rare in tadpole guts at all sites. Secondly, tadpoles from sites with inhibitors did not contain more cells on average than those from sites without inhibitors. In fact, we often found contradictory results. Tadpoles from Site 3 of Biesterfeldt *et al.* (1993), for example, had the highest density of *Prototheca* in tadpole guts even though water from this site was not inhibitory. In contrast, water from Site 5 was very inhibitory even though tadpoles had one of the lowest densities of *Prototheca* cells of any population sampled. Overall, the extent to which water from natural sites inhibited the growth of test animals was independent of *Prototheca* density in wild-caught tadpoles, with *Prototheca* density explaining only 2% of the variation in growth rates of test animals relative to controls. When tadpoles from the sites were returned to the laboratory and crowded for 72 hr, virtually all populations produced inhibitors even though levels of *Prototheca* did not increase in tadpole guts. Collectively, these trends led us to suspect that *Prototheca* was not functioning as an effective density-dependent regulating agent in most populations, and that other growth-inhibiting agents may have contributed to the inhibitory qualities of water collected from certain wood frog breeding sites.

Whether growth-inhibiting agents other than *Prototheca* contribute to interference competition in natural tadpole populations is unknown. In a general sense, we know that every vertebrate species has a wide array of faecal-borne pathogens, parasites, and disease agents that can slow growth or cause weight loss (certainly, the number in well-studied species such as humans must number over a hundred!). Consequently, I strongly suspect that the number of water-borne agents that affect tadpole growth is far greater than the number documented to date. Our studies were not designed to determine the specific agents causing growth inhibition. However, they do provide evidence which suggest that multiple agents may be involved in growth inhibition in natural populations.

Experiments conducted by Griffiths, Edgar & Wong (1991) under semi-natural conditions suggest that agents other than *Prototheca* may be involved in inhibiting growth of tadpoles. In one sample taken during the first week of their experiment, for example, *Prototheca* was not evident in tadpole faecal samples even though tadpoles were stunted. After four weeks, *Prototheca* occurred in low-to-moderate numbers in the faeces of stunted tadpoles. These observations along with data from our own studies suggest to me that faecal-borne agents in addition to *Prototheca* may be involved in growth inhibition under natural or semi-natural conditions. *Candida* is not a likely candidate since there is no evidence that it reaches high densities in tadpole faeces under natural conditions. However, bacterial and viral infections could be important and would be impossible to detect using standard microscopic examination of faeces. Perhaps the tendency of researchers to focus too narrowly on *Prototheca* has led us to ignore other faecal-borne, growth-inhibiting agents. A well-trained microbiologist might surprise us if she or he were to examine the growth inhibiting capabilities of the array of faecal-borne bacteria that inhabit tadpole intestines.

My scepticism should not downplay the important contributions that Beebee, Griffiths, and their colleagues have made in understanding the importance of *Prototheca* in regulating natural populations of tadpoles (see summaries in accompanying articles). Their work has provided much support for the argument that *Prototheca* may be an important density-dependent regulating agent in some (perhaps a small percentage) of amphibian populations. Wong, Beebee & Griffiths' (1994) recent discovery of two natural populations of tadpoles with high densities of *Prototheca* in their guts is significant. If they can further demonstrate that water from these sites is inhibitory, and that *Prototheca* is acting in a density-dependent manner to regulate these populations, they will be the first to demonstrate convincingly the ecological relevance of this phenomenon in nature. Given the experiences that my research group has had, I remain sceptical in pronouncing *Prototheca* the primary agent that is responsible for the growth-inhibiting properties of water that we have collected from anuran breeding sites.

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