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**TADPOLE GROWTH: IS THERE
AN INTERFERENCE EFFECT
IN NATURE?**

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The effects of crowding on growth-suppression in tadpoles have been the subject of laboratory studies for more than thirty years. Ever since the pioneering work of Richards (1962) and others, a unicellular organism has been implicated in an interference-type of competition that seems to operate on tadpoles of many different anuran species. Only recently, however, have intensive efforts been made to try and discover the mechanism of this process and, most importantly, whether it occurs in the field as well as in the laboratory. These new results are, at first sight, somewhat confusing and possibly contradictory.

In two recent papers (Petranka, 1989; Biesterfeldt, Petranka & Sherbondy, 1993), Petranka's group has reported experiments carried out at natural sites used by various anurans but especially the southern leopard frog *Rana utricularia* and the wood frog *R. sylvatica* in the USA, including studies with water taken from these sites and subsequently transferred to the laboratory. Broadly speaking, the experiments indicated, or were interpreted to indicate, that: (1) even in ponds with very high densities of tadpoles, it was rare (<25% of samples) for water from those sites to cause growth inhibition of other (target) tadpoles. This was true whether experiments were carried out *in situ*, or after transfer of water back to the laboratory. And (2), when growth inhibition was observed in field samples, the unicellular organism implicated as the crucial mediator in laboratory trials was probably not responsible.

By contrast, growth inhibition of small *Bufo calamita* tadpoles by large larvae of *Rana temporaria* has been demonstrated in the UK by Griffiths and his colleagues (Griffiths, 1991; Griffiths, Edgar & Wong, 1991; Griffiths, Denton & Wong, 1993) using replicated outdoor ponds which closely mimicked many natural ones used by these species. Although variable in degree, an interference-type mechanism was commonly observed and so was production of substantial numbers of the implicated unicells in the tadpole faeces. Studies by my group (Beebee, 1991; Beebee & Wong, 1992; Wong & Beebee, 1994) have confirmed that the organism involved is an unpigmented alga (genus *Prototheca*), and that purified cells cause growth

inhibition in small tadpoles under laboratory conditions by diverting them away from higher-quality food sources. More recently, we have also found that these cells were produced in large quantities by tadpoles in two natural ponds where densities were high, though the water from these ponds was not tested for growth inhibitory properties (Wong, Beebee & Griffiths, 1994).

Can these disparate results be reconciled? With respect to the significance of interference competition in the field, two aspects may be important: the availability and type of food consumed by tadpoles, and the abundance of *Prototheca* pathogens. Most laboratory experiments, and some of the replicated ponds used by Griffiths' team, received an artificial food (dried vegetable matter in pellet form). Since *Prototheca* seems to act by diverting tadpoles from alternative food sources, its efficiency in causing growth inhibition may be critically related to the type of food it is competing with. Perhaps it is much less successful when pitted against natural periphyton. Furthermore, we have also shown (Wong, Beebee & Griffiths, 1994) that a bacterial pathogen of *Prototheca* is widespread in natural pond silts. This pathogen is able to reduce *Prototheca* numbers drastically within a few days, and when present as an accidental contaminant can have similar effects in laboratory studies.

All of this means I share Petranka's view that interference competition may be less likely in natural than in laboratory situations. However, unlike Petranka I believe that the evidence clearly implicates *Prototheca* as a causative agent when this type of competition does occur, even in the field. Aside from the published evidence on this matter, and our findings of protothecans at high abundance in natural ponds, the data of the most recent paper by Petranka's group (Biesterfeldt *et al.*, 1993) gives some support to this hypothesis. Although the authors interpreted their data to mean that growth inhibition was caused by agents other than *Prototheca*, there is in fact an interesting relationship if the data in their Tables 2 and 3 are analysed. Thus, the numbers of *Prototheca* cells in fresh, wild-caught tadpole guts (Table 2) were negatively correlated with the arcsin-transformed percentages of growth relative to controls that can be calculated for the "target" tadpoles of Table 3. Both sets of data are normally distributed by the Shapiro-Wilk test and $r = -0.770$, $df = 6$, $P < 0.05$. Although there was no relationship between growth inhibition and *Prototheca* numbers in tadpoles kept for three days to condition water, this is not especially surprising. Feeding will have purged them of many cells (passage time, at least in *Rana temporaria* larvae, is >10 hours), and it is these which, in the conditioned water, probably caused the growth inhibition documented in Table 3. It is very likely that *Prototheca* numbers would have risen again in these tadpoles had they been kept longer; single cell-division times for these algae are usually about 24 hr in our ex-

perience, depending upon temperature. There was also almost a significant relationship, in Biesterfeldt *et al.*'s data of Tables 1 and 2, between *Prototheca* numbers in wild-caught tadpoles and tadpole mean local density per square metre of pond (a more meaningful measure than per cubic metre for a sediment organism of this kind); $r = 0.654$, $df = 6$, $P > 0.05$.

It is quite likely, therefore, that at least in site 2 of the Biesterfeldt *et al.* (1993) study *Prototheca* was mediating growth inhibition. This looks much less probable for the other two "inhibitory" sites, 5 and 6. However, the method used (assaying water taken from the sites) does not distinguish between inhibition due to interactions between tadpoles, and inhibition of growth that might be quite unrelated to tadpole products. Water samples from these ponds may have contained thermolabile toxins/inhibitors from other sources, and thus have nothing to do with competition between tadpoles. An interesting control would have been to screen water from a series of randomly-chosen ponds without any tadpoles at all.

Finally, bioassays in the Biesterfeldt *et al.* (1993) study were carried out in the presence of excess food and with only a single (initial) water addition. In our experience these conditions minimise *Prototheca*-mediated interference competition, which is more severe under laboratory conditions when food supply is limiting (Beebee, 1991; Griffiths, Denton & Wong, 1993) and may be weak if *Prototheca* is not continually replenished (as would of course occur in the wild). Growth inhibitors may therefore have been more prevalent than the bioassays revealed. Although *Prototheca* numbers in wild-caught tadpoles were generally much lower than those seen in laboratory stocks, this may not relate in any simple way to an effect on growth inhibition because *Prototheca* acts by resource diversion (and this might happen even at quite low cell numbers in faeces) and not by direct competition for nutrients within the tadpole guts (Beebee & Wong, 1992).

So where do we go from here? In my opinion, there are at least two areas which merit further research. Firstly, we need to identify what it is that makes interference competition happen in the field in those (possibly rare) places where it does occur. Is there something unusual about the natural food supply, or the abundance of *Prototheca* pathogens? Growth rates of *Bufo calamita* larvae in natural ponds are usually limited by food availability (Banks & Beebee, 1988), and this might make them especially susceptible to *Prototheca*-mediated growth inhibition; but in replicated pond trials, interference competition was more marked at high rather than at low food levels (Griffiths, Denton & Wong, 1993). This paradox needs further study. Secondly, the significance of overall competition (i.e. exploitation + interference) needs more attention both in the field and the laboratory. It is, after all, this holistic scenario that tadpoles nor-

mally face and in the case of tadpoles the distinction between the two types of competition is blurred; both are undoubtedly influenced by the quantity and quality of food available.

Despite some apparent discrepancies between recent studies, I am optimistic that within a few years we should have a much better idea about what really goes on in amphibian nurseries.

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