

DETERMINING COMPETITION MECHANISMS IN TADPOLE ASSEMBLAGES

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Amphibians and reptiles have proved to be popular organisms in competition studies, and have played an important role in influencing competition theory. Testing for interspecific competition requires the demonstration of a negative effect of one species on another. This may take the form of a shift in the distribution, or a decline in population size, of the inferior competitor. The practical demonstration of competition may therefore require long-term study. In the short term, though, variables such as growth and survival may provide an adequate reflection of competitive effects. Such variables are readily measured in small, rapidly growing animals such as tadpoles. The vast majority of studies, however, have treated competitive interactions between species as an ecological 'black box'; very few workers have attempted to unravel those mechanisms underlying the observed effects.

Traditionally, competition mechanisms have been divided into those concerned with 'exploitation' and those concerned with 'interference'. Exploitation competition reflects differences between species in their ability to utilize essential resources. One competitor will suffer because it will be deprived of resources, such as food or hiding places, by another superior competitor. The most overt form of interference competition is direct physical interaction between individuals (e.g. aggression), with the outcome of such contests often depending on the relative sizes of the competitors. A more subtle form of interference is the release of molecules or cells which may be toxic to inferior competitors, or may exert a negative effect on growth and survival via some other pathway. Interference mechanisms are unlikely to evolve unless resources are sufficiently scarce, and those based on the release of toxic chemicals or inhibitory cells are rare in nature (Schoener, 1982, 1983). The challenge for herpetologists is to open up the black box and tease apart the role of different mechanisms under field conditions.

Despite the flurry of work on tadpole growth inhibition between the 1950's and 1970's (e.g. Richards 1958, 1962; Rose, 1960; Licht, 1967; Heusser, 1972; Steinwascher, 1979a,b) unequivocal identification of the inhibitor involved has had to await the develop-

ment of the microbiological tools of more recent years. A combination of ultrastructural, cytological, nutrient assimilation and immunological methods has firmly established *Prototheca richardsi* as the mediator of growth inhibition, at least in laboratory cultures of British anurans (Beebee, 1991; Wong & Beebee, 1994). As discussed in the previous essays by Beebee (1995) and Petranka (1995), the next stage is to determine the importance of *Prototheca*, or possibly other interference mechanisms, as mediators of growth inhibition in the field.

The evidence for *Prototheca* as a mediator of competition in natural populations of tadpoles can be summarised as follows:

- (1) In laboratory bioassays, tadpoles treated with >98% pure *Prototheca* isolated from tadpole faeces suffer growth inhibition. Testing of other fractions from tadpole culture water fails to produce the same effect (Beebee, 1991).
- (2) In semi-natural replicated ponds, tadpoles treated with the faeces of superior competitors are inhibited. The faeces of both 'donor' and 'recipient' tadpoles in these experiments contain *Prototheca* (Griffiths, Edgar & Wong, 1991).
- (3) In natural ponds used by anuran populations, *Prototheca* has been detected in tadpole faeces and pond sediments. The density of *Prototheca* in pond sediment shows a seasonal trend related to the presence of tadpoles. In natural ponds not used by anurans no *Prototheca* have been detected at any time of the year (Wong, Beebee & Griffiths, 1994).

Petranka (1995) acknowledges that competition in tadpoles can be mediated by a faecal-borne inhibitor under natural conditions, but argues that this mechanism is unimportant in nature and that *Prototheca* is unlikely to be the main organism involved. The evidence that he and his co-workers produce for this is as follows:

- (1) When water taken from natural ponds containing high densities of tadpoles is tested for inhibitory properties, positive results are obtained at only a few sites (Petranka, 1989).
- (2) The numbers of *Prototheca* in the guts of tadpoles taken from natural ponds is not clearly related to the growth inhibition observed (Biesterfeldt, Petranka & Sherbondy, 1993).
- (3) Even in those tadpoles which are inhibited and show relatively high numbers of *Prototheca*, the number of cells observed in the gut is too low to explain the growth inhibition seen (Biesterfeldt *et al.*, 1993).

There is agreement between both schools in that (1) *Prototheca* mediated inhibition may be more prevalent in simplified laboratory systems than in the field, and (2) that the role of *Prototheca* in the field may be subservient to exploitation competition. As Petranka (1995) points out, apparently disparate results may be a reflection of differences in methodology.

So what experiments are necessary to show unequivocally that *Prototheca* is, or is not, an agent within tadpole faeces that causes growth inhibition under natural conditions?

There seem to be two basic approaches to the problem. The first is that already taken by Petranka and his co-workers, where water taken from natural tadpole ponds is tested for inhibitory properties. At those ponds where inhibitors are thought to be present, further fractionation and testing of the water and/or sediment needs to be conducted to isolate the organisms or molecules concerned. Wong *et al.* (1994) have described two natural ponds which had high densities of both tadpoles and *Prototheca*. Petranka (1995) suggests that if water from these sites was shown to be inhibitory then this would be the first convincing case of *Prototheca* acting as the mediator under natural conditions. However, to test the hypothesis that multiple agents are involved the same water would have to be carefully screened and tested for other putative inhibitors.

The second approach would be to dose natural populations of tadpoles with *Prototheca* isolated from the faeces of superior competitors, themselves raised under natural conditions. In our replicated pond experiments we have gone some way towards achieving this by showing that *Bufo calamita* treated with *Prototheca*-laden faeces from *Rana temporaria* were inhibited (Griffiths *et al.*, 1991). Petranka (1995) suggests that some other component of the faeces may have been responsible for the reduced growth. We have not attempted to test tadpoles by dosing with pure *Prototheca* in replicated ponds for purely practical reasons. If a system can be established for isolating *Prototheca* in sufficient numbers from the faeces of tadpoles raised under natural conditions, then it may be possible to attempt such an experiment in the future.

A further problem lies in identifying the importance of growth inhibitors relative to other exploitation and interference competition mechanisms in nature. Certainly, tadpoles are frequently food-limited in natural ponds which suggests that exploitation competition for food may be most important (e.g. Seale, 1980). However, ponds are dynamic environments, and the abundance and quality of the resources they contain may change rapidly over time. Temporary ponds may deteriorate as nutrients are depleted by animal and plant growth, and then exported from the system as tadpole metamorphosis occurs (Wilbur & Alford, 1985; Morin, Lawler & Johnson, 1990). There is also considerable variability between ponds in the microhabitats and food that they support. As Beebee (1995) points out, the effectiveness of *Prototheca* as a growth inhibitor seems to depend on tadpole food supply, and the variability of this resource between ponds could explain why Petranka (1989) found evidence of growth inhibitors in some ponds but not in others. In replicated ponds we have shown that *Bufo calamita* tadpoles which have access to *Rana temporaria* faeces

but are otherwise physically separated from the latter, display a level of inhibition which is intermediate between control (no *R. temporaria*) and fully interacting (*R. temporaria* + *B. calamita*) treatments (Griffiths *et al.*, 1991). This implies that exploitation and/or direct interference competition supplements that mediated by growth inhibitors. This approach could be extended to natural ponds by performing food manipulation experiments using tadpoles raised in enclosures. Treating tadpoles with various combinations of food supplements and growth inhibitors could shed further light on the relative importance of different competition mechanisms. It would be necessary to perform such manipulations on a wide range of ponds (i.e. encompassing both 'resource-poor' and 'resource-rich'), and at the same time collect baseline data on natural food resources contained within the enclosures.

The role of *Prototheca* and/or other agents which mediate competition in tadpole assemblages is unlikely to be resolved overnight. The lack of realism inherent in many rigorous experiments performed in the laboratory will continue to confound extrapolation to the field; the lack of rigour inherent in pond-based experiments will continue to confound the unequivocal identification of inhibitors in natural systems. The essays by Beebee (1995) and Petranka (1995) highlight the value of both approaches in experimental ecology. They also emphasize that some problems cannot be resolved in the short-term by a single, clean-cut experiment. A steady accumulation of evidence, gleaned from both the laboratory and the field, will ultimately lead to a resolution of the problem.

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