Evidence for the induction of interference competition between anuran larvae in plastic pond cages

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Abstract. We recently showed that growth inhibition of *Bufo bufo* larvae by those of *Rana temporaria* in garden ponds only occurred when the larvae were confined in plastic cages. We therefore carried out further experiments to investigate the mechanism of this growth inhibition. *Bufo bufo* larvae were raised in mesh cages, plastic cages or plastic cages with supplementary food either alone or in the presence of *R. temporaria*. Three lines of evidence suggested that competition in the plastic cages had an interference component: firstly, growth inhibition of *B. bufo* by *R. temporaria* was only seen in plastic cages despite the fact that these cages supported normal growth rates of *B. bufo* in the absence of *R. temporaria*; secondly, food supplementation in plastic cages did not reduce the extent of *B. bufo* growth inhibition; and thirdly *Anurofeca (= Prototheca) richardsi* production was high in plastic cages but not in other treatments and correlated negatively with subsequent *B. bufo* larval growth. Plastic cages supported a different trophic web from other treatments and biotic factors seem the most likely regulators of *A. richardsi* abundance in these pools.

Introduction

Among the model systems used to investigate competition as a structuring force in natural communities, the study of interactions between anuran larvae has proved particularly popular. These organisms regularly form complex guilds which can easily be reconstituted in the laboratory or artificial ponds, and have readily quantifiable fitness attributes including growth rate, development time and size at metamorphosis. Largely for these reasons, intra- and interspecific competition among larval anurans have been an area of research interest for several decades (e.g. DeBenedictis, 1974; Wilbur, 1982; Warner et al., 1993; Beebee, 1996). One interesting aspect of competition that has received occasional attention over this period is the extent to which resource and interference mechanisms are involved (e.g. Richards, 1958; Steinwascher, 1979; Griffiths, 1991).
Although resource competition is often assumed to be the most important, relatively few studies have attempted to distinguish the two processes. Furthermore, there seems to be a substantial discrepancy between events in the field and those in the laboratory. Interference competition under controlled conditions in the latter environment has been demonstrated many times (e.g. Richards, 1962; Beebee, 1991). The mechanism is apparently complex, and usually involves unicellular eukaryotes passaged in tadpole faeces. One such unicell is *Anurofeca (= Prototheca) richardsi* (Baker et al., 1999), an indigestible organism which proliferates in the guts of large larvae and, when excreted in their faeces, apparently diverts small larvae from higher-quality food resources into coprophagy (Beebee and Wong, 1992). Similar effects have also been demonstrated in replicated pond experiments (Griffiths, 1991; Griffiths et al., 1993). However, interference competition seems to be much rarer in natural ponds (e.g. Morin and Johnson, 1988; Petranka, 1989; Biesterfeldt et al., 1993).

In Britain *Rana temporaria* is widely syntopic with *Bufo bufo*, but the former species breeds earlier in the year and its larvae are therefore usually larger than those of the latter. We have investigated interactions between these two species in garden pond environments. Although artificial, these ponds now constitute the most widespread and abundant breeding sites for anurans in much of Britain, following widespread losses of freshwater habitats in rural areas (Beebee, 1979, 1981). Remarkably large amphibian populations develop in and around garden pools. Despite high initial densities of spawn and larvae, however, competition is not an important structuring force in assemblages of *R. temporaria* and *B. bufo* larvae inhabiting small garden fishponds in southeast England. This is at least partly because predation levels on larvae are very high, and larval numbers fall very quickly after hatch (Baker and Beebee, 1997). However, growth of *B. bufo* in plastic-coated cages immersed in the same ponds was substantially inhibited by *R. temporaria* and *Anurofeca* was found at high titres in these plastic cages (Baker and Beebee, 1997). In all these cage trials, both species were maintained at densities comparable with those in the surrounding ponds.

We therefore set out to probe the mechanism of the growth inhibition apparently induced in plastic cages, and what features of the plastic-contained microenvironment promoted competition and *Anurofeca* accumulation. In particular, we hypothesised that increased food resources in plastic containers should relieve growth inhibition if only resource competition occurs. By contrast we predicted much less effect of elevated food levels if interference competition was a significant component of the interactions.

**Methods**

**Study site**

The experiment was conducted in a long-established garden pond in Brighton, UK. This pond had been used in previous studies (Baker and Beebee, 1997) and was known to have
breeding populations of both *R. temporaria* and *B. bufo*. It was well-vegetated and also contained goldfish (*Carassius auratus*). With a perimeter of 22.6 m and an approximate volume of 1800 litres, it was also the largest garden pond known in the area.

**Tadpole density, survival and growth in the study pond**

Spawn clumps of *R. temporaria* were counted within a week of laying, during March 1997. Egg numbers were estimated by multiplying clump number by mean number of eggs per clump (Cooke, 1975), and the density of eggs per litre was calculated. Despite spawning in this pond for the previous five years, *B. bufo* did not spawn there in 1997. Approximately 4,500 *B. bufo* hatchlings from another pool were therefore added to the pond 22 days after *R. temporaria* spawn hatched, generating an initial density similar to that seen in previous years (Baker and Beebee, 1997). Mark-recapture using tail fin clips (Banks and Beebee, 1988) was carried out on *R. temporaria* and *B. bufo* larvae fortnightly from the second week after hatching to estimate numbers using a simple Bailey’s index (Southwood, 1966) and thus survival rates (Baker and Beebee, 1997). Tadpole growth rate was monitored by measuring the body length (snout-vent) of ten arbitrarily chosen free-swimming individuals of each species on a weekly basis.

**Experimental treatments**

Cages were placed in the pond in February 1997. Each cage was an open-topped rhomboid with a sloping bottom to follow the pond contours and thus allow natural thermoregulation by the tadpoles. The cages consisted of a stainless steel frame wrapped in polyethylene 2 mm diamond mesh (Netlon, UK). Dimensions were 40 cm (front width) × 45 cm (length) × 20 cm (rear width) × 40 cm (front height) × 25 cm (rear height). Eight of the cages were coated on the outside with 500-gauge clear plastic bags sealed with waterproof tape. All cages were immersed sufficiently as to contain 20 litres of pond water, and sediment and vegetation were added to simulate conditions in the pond outside.

Despite selecting the largest suitable pond available for this study, its relatively small size imposed a constraint on the number of cage trials that could be accommodated. However, because of the precise nature of the hypothesis under test we were able to dispense with some treatments that would have been necessary for a fully orthogonal design. Furthermore all of the treatments were only duplicated, the greatest level of replication possible within the pond volume available. We nevertheless expected that this level of replication should be adequate to demonstrate the substantive differences in growth rates between treatments anticipated on the basis of our previous study (Baker and Beebee, 1997). The experimental design is summarised in table 1. *Bufo bufo* was either alone (treatments 1-3) or with *R. temporaria* as a potential competitor (treatments 4-6). The three treatments in each of these two groups were: mesh cages (treatments 1 and 4) in which larval growth rates of both species were expected to mimic rates in the surrounding pond; plastic-coated cages (treatments 2 and 5) were employed to promote interference
Table 1. Experimental treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cage type</th>
<th>Species present</th>
<th>Food added</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>mesh</td>
<td><em>B. bufo</em></td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>plastic</td>
<td><em>B. bufo</em></td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>plastic</td>
<td><em>B. bufo</em></td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>mesh</td>
<td><em>R. temporaria + B. bufo</em></td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>plastic</td>
<td><em>R. temporaria + B. bufo</em></td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>plastic</td>
<td><em>R. temporaria + B. bufo</em></td>
<td>+</td>
</tr>
</tbody>
</table>

competition and *Anurofeca* production; finally, 10 g of blanched lettuce were added to half of the plastic cages (treatments 3 and 6) every week between mid-March and late May as a food supplement. This treatment was to test whether or not growth inhibition of *B. bufo* in plastic cages was relieved by extra food. Lettuce was chosen because it could be suspended above the bottom sediment and should therefore not promote coprophagy, a behaviour that stimulates proliferation of *Anurofeca richardsi* (Beebee and Wong, 1992). Natural densities of *R. temporaria* and/or *B. bufo* larvae (i.e. densities comparable to those in the surrounding pond) were maintained in all treatments. One week after hatching, *R. temporaria* larvae were placed into cages at natural pond densities estimated from egg numbers (124 tadpoles per litre; 2,480 per cage). Seven day-old *B. bufo* larvae were placed in treatment cages at 2.5 tadpoles per litre (50 per cage) three weeks after *R. temporaria* had hatched. Densities of both species were decreased every fortnight to mimic those in the natural pond, as calculated by mark-recapture. Larvae were removed from the cages at random to prevent size bias, and released into the main pond. This was considered essential to avoid generating widespread competitive effects by keeping larval densities unrealistically high. From week 6 to week 10, however, densities were left as those measured on week 5 (i.e. *R. temporaria* 1 tadpole/litre; *B. bufo* 0.25 tadpole/litre) to ensure there were enough tadpoles for measurement. Five randomly selected *R. temporaria* and *B. bufo* larvae from each treatment cage were measured (snout-vent) on a weekly basis. The experiment was terminated when metamorphosis of *R. temporaria* was imminent.

*Anurofeca titres*

Faecal samples from five tadpoles of each species, from each treatment cage and from the main pond, were collected every week from 14 to 63 days after hatching (Baker and Beebee, 1997). The faeces of the five tadpoles in each sample were shaken vigorously together, then centrifuged and resuspended in 5 ml of sterile water. 50 μl samples were placed on a haemocytometer and the numbers of *Anurofeca* cells present were counted under a phase contrast microscope (Beebee, 1991). This method has a minimum detection limit of approximately $10^3$ cells produced per tadpole per hour. Total production of *A. richardsi* per cage was estimated as the product of mean *A. richardsi* numbers produced
per tadpole per hour (for each species separately in mixed treatments) and total numbers of each species in the cage.

Environmental conditions in treatment cages

A number of environmental variables were recorded within each of the treatment cages and in the main pond every other week. Temperatures were measured using max-min thermometers submerged 5 cm below the water surface. Oxygen levels were measured using a YSI Model 58 Oxygen meter, precalibrated in tap water oxygenated for 18 hours with an aquarium pump. The probe was left to equilibrate 5 cm below the water surface in the cages for 5 minutes before oxygen measurements were taken. Absorbance and conductivity measurements were made on 20 ml samples of water taken from each of the sample cages and the main pond. These samples were withdrawn before other measurements were made, to avoid mixing sediment into the water sample. Absorbance at 550 nm was measured in a Pye Unicam SP6-550 spectrophotometer. Conductivity was measured using a WPA CM25 conductivity probe.

Data analysis

In all cases, the means of measurements within each replicate cage were treated as individual datum points. Analysis of variance (ANOVA) for repeated measures was carried out on the mean body size data of *B. bufo* from sampling periods between days 29 and 64. A one-way ANOVA was then conducted at specific time points across all treatments. Tukey tests were carried out on these ANOVAs to assess which specific differences were significant (Fowler and Cohen, 1990). Similar analyses were performed on those environmental measurements taken in the treatment cages which showed any indication of variation.

The frequency of *Anurofeca* was compared between the three main types of treatment (i.e. mesh, plastic and plastic with food) using G-tests (Fowler and Cohen, 1990). Differences in numbers of *A. richardsi* produced per tadpole per hour were compared between appropriate pairs of treatments (e.g. with or without food addition; single or mixed-species) using Mann-Whitney tests at a single time point. Relationships between total *A. richardsi* production per cage and mean size of *B. bufo* larvae were estimated using Spearman rank correlation. All statistical analyses were performed with MINITAB or SPSS software packages.

Results

Tadpole survival in the main pond

There were an estimated 222,300 viable *R. temporaria* eggs laid (about 124/litre) in the study pond. Five weeks after hatching approximately 1,490 larvae (< 1% of the
original eggs) remained, and densities were < 1/litre (fig. 1). At least some R. temporaria metamorphosed from the pond in the ninth week after hatching, though this was not quantified. Two weeks after the introduction of B. bufo hatchlings, their densities had fallen to 0.5/litre. Bufo bufo was undetectable in the main pond six weeks after introduction and metamorphosis of B. bufo was not observed. Survival estimates in the main pond were used to calculate tadpole density changes during development, and thus to adjust densities in the treatment cages to keep them similar to the natural ones. In the case of B. bufo, however, larvae were maintained in the cages at 0.5 per litre after they disappeared from the main pond until the end of the experiment.

**Treatment effects on tadpole growth**

There were significant treatment effects on the growth of B. bufo over time related to cage type (mesh, plastic or plastic + food), presence or absence of R. temporaria and interactions between these variables (figs 2A, B; table 2). One-way ANOVAs at different time points during the experiment indicated more clearly the nature of these differences. Trends were consistently significant between day 36 and day 57, corresponding to the main growth period, though of course separate analyses at these times cannot be considered as independent of each other. To take a single example, therefore, on day 57 the food-supplemented B. bufo in plastic cages were significantly larger than those in all the other treatments. Bufo bufo in plastic cages without food supplement were also larger than those in the same treatment but with R. temporaria present (Tukey tests all at $P < 0.05$, based on

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**Figure 1.** Survival of *Rana temporaria* and *Bufo bufo* in the study pond. ●, R. temporaria; ○, B. bufo. Error bars show standard deviations of estimates.
on one-way ANOVA $F_{5,6} = 42.99, P < 0.001$). By contrast, there were no significant differences between any other treatment combinations (all Tukey tests $P > 0.05$). Among other things this indicates that *B. bufo* in mesh cages were not reducing their growth rate in response to cues from predators present in the pond, a potential complicating effect which could result in tadpoles growing more quickly in plastic cages isolated from such cues. Some growth retrieval of *B. bufo* occurred late in the experiment as *R. temporaria* densities were reduced in the cages to match those in the pond outside. For most of the experiment, however, growth retardation of *B. bufo* in plastic cages with *R. temporaria*, relative to controls in the absence of competitor, was substantial. Thus on day 57 *B. bufo* with *R. temporaria* were on average about 71% as large as uninhibited controls (all in the absence of food supplement), while in the presence of food they were about 66% as large. Although free-swimming *R. temporaria* were on average slightly larger than those in treatment cages toward to end of the experiment (data not shown), there were no significant differences in the growth of *R. temporaria* under any of the experimental conditions within the statistical power available to test them (fig. 2C).

*Anurofeca* titres in faeces expressed as mean number excreted per tadpole per hour, averaged over both species, are shown for the three cage categories in figure 3A. Both species contributed significantly to the late production peak between days 43 and 64, but the small peak around day 29 was entirely from *R. temporaria*. The frequency at which *Anurofeca* occurred in faecal samples was significantly higher in the plastic treatments compared to the mesh treatments and free-swimming controls ($G_{\text{adjusted}} = 323, df = 2, P < 0.01$). Indeed, anurofecan cells were never encountered in faeces from tadpoles in mesh cages. There was an apparent tendency after day 50 in plastic cages for food-supplemented larvae to produce more anurofecan cells than the smaller, unsupplemented ones. Even at the peak time of *A. richardsi* production (day 57), however, this difference was not quite significant (Mann-Whitney $W = 19.5, P = 0.067$). There was no indication that larvae in mixed-species plastic cage treatments produced more anurofecans than those (*B. bufo*) reared alone.

When total *Anurofeca* production was calculated per cage, rather than per tadpole, an early peak of *Anurofeca* production was evident in mixed-species treatments at around day 29 (fig. 3B). This was essentially due to the large numbers of *R. temporaria* still present in mixed-species cages at this time, and there was no detectable contribution from the very small *B. bufo* larvae. Within the plastic cage treatments (i.e. those in which *A. richardsi* production and growth inhibition of *B. bufo* were observed), there was a strong negative correlation between production of *A. richardsi* in cages at day 29 and the mean size of *B. bufo* larvae on each and every subsequent day ($r_s = -0.943$ on day 36; $-0.759$ on day 43; $-0.829$ on day 50; $-0.954$ on day 57; and $-0.804$ on day 64. $n = 8, P < 0.05$ in all cases).

There were no significant differences in water temperatures, oxygen levels or conductivities between the pond, mesh and plastic treatments. Absorbance at 550 nm of water from plastic treatments was however significantly lower (mean 0.007) than that in the
Figure 2. Effects of treatments on larval growth. A and B: *B. bufo*; ●, free-swimming controls; ■, species alone in mesh cages; ▲, species alone in plastic cages; △, species alone in plastic cages with food supplement; □, species with potential competitor in mesh cages; ▼, species with potential competitor in plastic cages; ◀, species with potential competitor in plastic cages with food supplement. C: *R. temporaria* (always with *B. bufo*); ▲, in mesh cages; ▼, in plastic cages; ◀, in plastic cages with food supplement. In all cases symbols represent means of replicate treatments, with standard deviations shown by error bars.
Competition between anuran larvae

Figure 2. (Continued).

Table 2. Repeated-measures ANOVA of *B. bufo* growth rates in cage treatments. Cage type refers to mesh, plastic or plastic with food supplement treatments.

<table>
<thead>
<tr>
<th>Variance</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>119.72</td>
<td>5</td>
<td>23.94</td>
<td>150.06</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cage type</td>
<td>11.48</td>
<td>10</td>
<td>1.15</td>
<td>7.19</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Presence of <em>R. temporaria</em></td>
<td>4.04</td>
<td>5</td>
<td>0.81</td>
<td>5.06</td>
<td>0.006</td>
</tr>
<tr>
<td>Interactions (Time × cage × <em>R. temporaria</em>)</td>
<td>4.95</td>
<td>10</td>
<td>0.49</td>
<td>3.10</td>
<td>0.024</td>
</tr>
<tr>
<td>Within + Residual</td>
<td>2.39</td>
<td>15</td>
<td>0.16</td>
<td>3.10</td>
<td>0.024</td>
</tr>
</tbody>
</table>

pond or in mesh treatments (mean 0.03), overall by a factor of more than fourfold (repeated measures ANOVA $F = 41.13$, df = 7, 2, $P < 0.001$). There was no significant correlation between daily Anurofeca titres and daily absorbance measurements, but both Anurofeca abundance and low absorbance measurements were clearly associated with the plastic treatments. There were no significant intercorrelations between any of the other environmental variables.
Figure 3. Effects of treatments on *Anurofeca* production. A: *Anurofeca* production per tadpole per hour. ○, average of all larvae in all plastic cages with food; ●, average of all larvae in all plastic cages without added food; ★, average of all larvae in mesh cages and those free-swimming. Values of 0 represent samples containing $< 1 \times 10^3$ *Anurofeca*/tadpole/hour. B: Average total *Anurofeca* production per plastic cage per hour by all tadpoles present, with standard deviations of duplicates shown by error bars. ○, *B. bufo* alone, no added food; ●, *B. bufo* alone, with added food; □, *B. bufo + R. temporaria*, no added food; ■, *B. bufo + R. temporaria*, with added food.
Discussion

The ease with which it was possible to induce competition at natural larval densities in an artificial microenvironment clearly poses the question as to whether similar circumstances might also arise in nature. This in turn could have significant implications for the organisation of amphibian community structures.

Mortality rates of *R. temporaria* and *B. bufo* in the pond outside the cages were high in 1997 as in 1996. Five weeks after hatching, *R. temporaria* densities had fallen by 98% relative to numbers of viable eggs and *B. bufo* densities had fallen by over 75% relative to the stocked density of hatchlings. At least in the case of *R. temporaria* this low survival was probably a result of intense predation by the fish present in the pond. High rates of predation are of course likely to overrule competition (e.g. Morin, 1983) and it seems very likely that this was occurring here. From weeks 3 to 9 after hatching the densities of both species in the pond were lower than those commonly used in competition experiments in the laboratory (e.g. Beebee, 1991). However, even at these densities both competition and *Anurofeca* production were induced in plastic cages placed within the ponds while no comparable effects were seen in mesh enclosures. Three lines of evidence indicate that interference mechanisms were probably occurring in the plastic-confined microenvironments.

Firstly, *B. bufo* larvae in plastic cages without added food grew as fast or faster than those in the other unsupplemented treatments (i.e. mesh cages and the pond itself). *Rana temporaria* grew equally well in all treatments. These results indicate that food supply in the unsupplemented plastic cages was at least as high as that available in the pond and in the mesh cages. However, growth inhibition of *B. bufo* by *R. temporaria* was evident in the plastic cages but not in other treatments. This is difficult to explain on the basis of simple resource competition.

Secondly, an important prediction of this study was that growth inhibition of *B. bufo* with food supplements should be less severe than that without such supplements if resource competition was the main cause of growth inhibition. The added food had a substantial effect on growth rates of *B. bufo*, almost doubling them when this species was alone in plastic cages, confirming that the ration chosen was sufficient to have a detectable effect. However, when *R. temporaria* were included, growth inhibition of *B. bufo* was at least as great when food was added as when there was no supplement. These results are therefore also not easily reconciled with simple resource competition.

Thirdly, *Anurofeca* was essentially absent from mesh cages and the main pond, but was found in all plastic cage treatments. However, *Bufo bufo* alone in plastic cages with food were larger than those in all other treatments despite producing titres of *Anurofeca* comparable, on a per tadpole basis, with growth-inhibited larvae. *Anurofeca* production as measured on this basis peaked quite late in development (after day 50). Probably of greater relevance to growth inhibition were the dynamics of total *Anurofeca* production in the treatment cages rather than production per tadpole. This gave a very different picture,
peaking at early times (around day 29) and at very high titres in the mixed-species cages due to the large numbers of *R. temporaria* then present. This large *Anurofeca* output coincided with the onset of growth inhibition in *B. bufo* tadpoles at the early developmental stages when they are most susceptible to interference effects (Heusser, 1972; Beebee, 1991).

The question remains as to why the microenvironment within plastic cages particularly favours *Anurofeca* production. Analysis of environmental variables between cages demonstrated few differences between treatments, but absorbance of water at 550 nm was significantly higher in mesh cages and the main pond than in plastic cages. This was apparently due to lower numbers of unicellular green algae in the plastic cages, which contained abundant zooplankton such as *Daphnia pulex* that feed upon such algae. High densities of goldfish in the main pond probably reduced zooplankton levels there, thus allowing green algae in the main pond and mesh cages to proliferate. From these results it appears that *Anurofeca* abundance was related to the nature of the trophic web, and in particular may have been inverse to the abundance of green algae. Laboratory tadpoles raised in tap water or pond water kept out of sunlight also produce large numbers of *Anurofeca*, but this organism can also occur at high titres in ponds when predation levels on larvae are low and tadpole densities remain high throughout development (Wong et al., 1994).

Wong et al. (1994) demonstrated that *Anurofeca* numbers remained stable in tap water but often declined when incubated in pond water. Biesterfeldt et al. (1993) also found lower rates of *Anurofeca* accumulation in the field than in the laboratory. Abiotic factors such as UV light and temperature were suggested as potential factors affecting *Anurofeca* accumulation. However, results from this and our previous studies (Baker and Beebee, 1997) indicate that abiotic factors such as UV light, temperature and oxygen level probably have little direct effect on *Anurofeca* accumulation. Taken together, our observations suggest that biotic factors probably play a major role in the control of *Anurofeca* levels. Evidently relatively minor manipulations can cause a transition between field conditions disfavouring *Anurofeca* production to conditions highly favourable to it, and in which competition between tadpoles is induced at relatively low population densities. It remains to be seen as to whether such conditions ever occur naturally in the field.

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**References**


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