THE EFFECTS OF COHORT STRUCTURE AND DENSITY ON LARVAL GROWTH AND DEVELOPMENT IN *ALYTES MULETENSIS*: IMPLICATIONS FOR CONSERVATION

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The Mallorcan midwife toad (*Alytes muletensis*) has a very extended breeding season, and the nature of competition between larvae in the torrent pools where it breeds is likely to change across time. Larvae commonly overwinter and grow to a very large size and new hatchlings will have to compete with these overwintered larvae under varying conditions of density. The effects of density and cohort structure (i.e. the presence/absence of large overwintered tadpoles) on the growth and development of hatching *A. muletensis* larvae were investigated in the laboratory using a factorial design. Large competitors, high densities and lower temperatures were all shown to suppress tadpole growth and development. Larger competitors were superior, especially the very large overwintered tadpoles. Whilst large size is advantageous, avoiding competition with overwintered tadpoles or high densities of tadpoles is probably much more important in determining size at – and timing of – metamorphosis. Because *A. muletensis* is an endangered species, knowledge of life history constraints can guide management of wild populations. The results are discussed in terms of potential optimal times to breed in light of the changing competitive environment.

*Key words:* amphibian, anuran, competition, density effects, tadpoles

INTRODUCTION

The Mallorcan midwife toad (*Alytes muletensis*) is an endangered species (Groombridge, 1994) and knowledge of life-history constraints can guide the management of wild populations. To date, however, there is little information on a fundamentally important part of its life cycle, namely the larval environment (but see Schley, 1996; Griffiths et al., 1998). In many anurans, the effects of competition in the larval environment can carry through to the juvenile and adult stages (Berven, 1990). A large tadpole will become a large metamorph which may have better physiological performance (John-Alder & Morin, 1990), become a larger adult or, together with early metamorphs, reproduce sooner, and may ultimately survive longer (Berven & Gill, 1983; Smith, 1987). Conversely, late metamorphosis will leave less time for terrestrial feeding and juveniles may not be able to accumulate enough energy to see them through the winter (Berven & Gill, 1983) unless they can compensate for poor aquatic growth by feeding rapidly on land (i.e. ‘catch up’ growth in species with extended juvenile periods, Halliday & Verrell, 1988). Larval competition can thus be the most important determinant of fitness at all stages of the life cycle.

*A. muletensis* larvae do not necessarily metamorphose at the end of a breeding season but sometimes overwinter as tadpoles which, by the beginning of the following season, have reached a very large size (65-88 mm long, Bush, 1993 and Lea, pers. obs). Therefore, new hatchlings (11-15 mm long) may have to compete for resources with these much larger tadpoles. In *Bufo woodhousii*, another prolonged breeder, tadpoles hatching late in the season must compete with larger conspecifics, and this suppresses the developmental rate and lowers the chances of survival of the smaller larvae (Woodward, 1987). The enormous size range found in *A. muletensis* and the very extended breeding season (5-7 months) may mean that the effects of competition with larger larvae could be considerable.

A high density of tadpoles can also affect fitness; usually prolonging development and increasing the variation and reducing the average size at metamorphosis, as well as reducing larval survival (Richards, 1958; Brockelman, 1969; Wilbur, 1977; Buskirk & Smith, 1991). Conversely, some authors have shown that an increase in density can be beneficial, actually enhancing growth (Beiswenger, 1975; Breden & Kelly, 1982) via ‘facilitation’, whereby many interacting tadpoles stir up more food from the benthos and individuals exhibit higher rates of feeding behaviour in larger groups.

Previous experiments with *Alytes* have produced contradictory results with respect to the effects of density on larval growth. There is some evidence that *Alytes* tadpoles grow better in crowded conditions than under conditions of lower density. *Alytes obstetricans* larvae reared in isolation grow and develop slower, show more variation in the timing of metamorphosis, and metamorphose at a lower size than tadpoles reared in small groups, thus indicating some facilitation in this species (Guyetant, 1970). To date there have been no papers
published on density effects in *A. muletensis*. This paper explores the potential consequences of competition in the larval environment for *A. muletensis*, looking at the effects of the size of competitors and the number of competitors on larval growth and development.

**MATERIALS AND METHODS**

Tadpoles were bred in captivity from wild Mallorcan adults that formed part of a captive breeding programme. They were measured and staged weekly for 98 days after hatching. There were two density conditions: ‘high’ (H) (eight larvae per container) and ‘low’ (L) (two larvae per container). There were also two cohort conditions: ‘all small’ (s) (all the larvae were small i.e. all hatched on the day that the treatments were started) and ‘half larger’ (l) (half the larvae were larger i.e. half were one-year-old tadpoles, over-wintered from the previous season). Thus, there were four treatments in total: ‘Hs’ (high density – all small), ‘Hl’ (high density – half larger), ‘Ls’ (low density – all small); and ‘Ll’ (low density – half larger; Fig. 1).

There were four replicates of each treatment (a total of sixteen replicates), which was the maximum number practicable due to constraints on laboratory space. The limited availability of hatchlings at any one time (because of the protracted breeding and small clutch size of this species) (clutch size = 11±3 eggs, Lea pers. obs.) meant that replicates 1 and 2 had to be started earlier (16 June 1997) than replicates 3 and 4 (started on 23 July 1997). The decreasing temperature later in the summer (water temperature at noon ranged from 22.1 to 16.4 °C over the course of the experiment) led to slower growth and development of the tadpoles in replicates 3 and 4 compared to replicates 1 and 2; this is referred to as the effect of ‘season’ in the analysis.

Tadpole length was measured using a V-shaped trough made from two rulers glued together at 45° and sealed (with a zero mm mark) at each end. Each tadpole (its mouth touched either end of the trough). Measure­ments were taken blindly to a precision level of 0.5 mm (Pearson’s r=0.99, df=18, P<0.001).

Development was mapped using the stages described for *A. obstetricans* (Cambar & Martin, 1959). The patterns of differentiation for metamorphosing *A. muletensis* and *A. obstetricans* larvae are exactly the same; the only differences are in rate and stage-specific size. The stages relevant to the experiment as categorised by Cambar & Martin (1959) were ‘IV5’ through to ‘IV15’. IV5 is the free-living tadpole upon hatching from the egg; IV15 describes the point at which the buccal denticles start to diminish and aquatic feeding behaviour consequently begins to alter. IV15 is easily determined, because at this point the hind limb bud elongates and gets its characteristic bending, and tadpoles have stopped increasing in length but have not yet started to reabsorb their tails. Thus IV15 was deemed an appropriate point to stop taking measurements.

The extended development in this species, together with the late start of the experiment relative to the onset of the breeding season, meant that many of the tadpoles had not completed metamorphosis by the end of the experiment. Because of this, a reference point – day 42 after hatching – was used to compare the size and developmental stage of the tadpoles under the different regimes. Day 42 was used because this marked the point where the fastest developing tadpole in the experiment reached stage IV15.

For each set of replicates hatchling tadpoles were drawn from a minimum of three clutches, each from a different female, in order to minimise any differences in genetic or maternal (egg quality) effects on growth and differentiation. Tadpoles were randomly selected for each treatment by collecting those that swam freely into its mouth. Measurements (temperature: F3.36=0.36; pH: F3.36=0.74; oxygen: F3.36=0.67; P>0.05 in all cases). Faeces were collected regularly from all the aquaria, squashed and diluted in a drop of distilled water, and examined under the microscope for the presence of *Anurofeca* (previously *Prototheca*; Beebee & Wong, 1992; Beebee, 1995). Food ration (35 mg of crumbled ‘Tetra Min’ fish food per tadpole per week) was calculated from trials measuring the maximum food clearance rate of the larger tadpoles, hence there was *ad libitum* food at all times. Tadpole density was determined in the high-density replicates by the maximum amount of food

![FIG. 1. Cohort and density treatments - see text.](image-url)
that could be applied to each aquarium before visible fouling of the water occurred. This quantity was then divided by the per capita ration and rounded down to give an estimate of what would be a suitable maximum density. Two tadpoles were used in the low density treatments to provide an adequate contrast, while limiting any possible negative effects on growth that may occur by housing tadpoles singly (e.g. a lack of facilitation).

**Statistical Analysis**

Only the 'small' tadpoles were measured as these were the 'targets' of competition. There were three variables used in the analyses:

**Variable 1.** Individual tadpole sizes at day 42.

**Variable 2.** Increment in size at day 42, calculated as: (individual tadpole size at day 42 - mean tadpole size in replicate at day 0) / mean tadpole size in replicate at day 0.

**Variable 3.** Mean developmental stage reached by day 42 in replicates 1 and 2 only (see below).

Three-way ANCOVA or ANOVA were used to determine whether the treatment effects (density, cohort and season) differed significantly for variables 1 and 2 respectively. The two analyses (ANCOVA on variable 1, ANOVA on variable 2) both measure the treatment effect on size at day 42 whilst controlling for size at day 0. Agreement between them therefore lends support to any conclusions.

Two-way ANCOVA was used to determine whether the effects of density and cohort differed significantly for variable 3. Although stage data are ordinal, according to the 'central limit theorem' means should be normally distributed even if the original data are not (Sokal & Rohlf, 1980). Only replicates 1 and 2 were analysed because most of the tadpoles in replicates 3 and 4 were at the same developmental stage at the end of the experiment. (By omitting replicates 3 and 4 from the analysis the data conformed to the assumptions of the ANCOVA).

The mean size of tadpoles in each replicate at day 0 was used as a covariate in the ANCOVAs, and also in the equation used to calculate variable 2, because individual tadpoles could not be identified during the experiment.

**RESULTS**

**Size at Day 42**

**Single effects.** Growth rate slowed dramatically as the summer progressed (Fig. 2, Table 1), and tadpoles in the later replicates (3 and 4) were much smaller than those in the earlier replicates (1 and 2) (effect of season: $F_{1,51}=38.9, P<0.001$). Seasonal affects apart, the presence of larger competitors had the most significant effect on tadpole size at day 42. Larvae were much larger in the 's' treatments (all small tadpoles) than they were in the 'i' treatments (with larger competitors) (effect of cohort: $F_{1,51}=8.78, P<0.01$). Fig. 2 also shows that there was far more variation in growth in the 'i' treatments than in the 's' treatments. (For high density treatments, the CV of 'i' treatments = 19.1%, whilst the CV of 's' treatments = 11.4%; mean sizes are 31.2 mm and 37.3 mm respectively). The next most significant single effect was density. At day 42, tadpoles were larger in the low-density treatments ('L') than they were in the high density treatments ('H') (effect of density: $F_{1,51}=7.53, P<0.01$). Starting size also had a significant effect on size at day 42, although this was less important than either density or cohort (effect of mean size at day 0: $F_{1,51}=4.42, P<0.05$).

**Combined effects.** Density had an effect regardless of season (there was no significant interaction between density and season: $F_{1,51}=0.91, P>0.05$). Tadpoles showed the same trend for both the early (1 and 2) and
TABLE 1. Results of ANOVA or ANCOVA on size, stage and increment at day 42. * P<0.05, ** P<0.01, *** P<0.001, NS P>0.05.

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![Graph](image)

FIG. 3. Interactions (for size, in mm, at day 42) between: a, density and season (density had an effect that was independent of season i.e. in both the early and late replicates tadpoles were larger under low densities than they were under high densities (Fig. 3a). There was an interaction between cohort and season with the ‘large’ cohort suppressing growth) but had no effect in replicates 1 and 2 (Fig. 3b). There was also an interaction between cohort and density (Fig. 3c) was much greater at high than at low density. High density had an independent effect on reducing mean size at day 42 without large competitors being present ('cohort small'). Also, there was no difference between the replicates with or without large competitors at low density (probably due to the small sample size of the low-density replicates), but there was a compound effect on growth suppression when large competitors were combined with high density.

No *Anurofeca* were found in the present study. However, they have been found in the faeces of captive-reared *Alytes muletensis* tadpoles before (R. Griffiths, pers. com.).

STAGE AT DAY 42

None of the tadpoles in replicates 3 or 4 had started to approach metamorphic climax by day 42, and all except for one individual were still at stage IV6 (that individual had only reached stage IV7, Fig. 4). Replicates 3 and 4 are not included in the following analysis as they introduce skew into the data set.

The only significant effect on mean stage was that of cohort (F1.3=11.16, P<0.05). The mean stage in the treatments without larger competitors was 9.3±1.09, which compares to 6.3±0.24 when larger competitors were present. The effect of the covariate ‘mean size at day 0’ was not significant (F1.3=1.9, P<0.05). The effect of density was not significant (mean stage: high-density treatments = 7.1±0.99, low-density treatments =
influenced by the fact that some tadpoles in Hs2 had started off at a relatively large size. 0.6
increments are
FIG. 5. Mean size increment at day 42. Units for mean size are mm; error bars show standard errors.

FIG. 4. Mean stage at day 42. Stages are IV5 to IV15 from Cambar & Martin (1959). Error bars show standard errors.

8.5 ± 1.17, \( F_{1, 52} = 2.0, P > 0.05 \). This lack of significant difference between the high and low-density treatments is influenced by the fact that some tadpoles in Hs2 had reached stage IV15 by day 42 (Fig. 4). It is noteworthy that tadpoles in Hs2 had started off at a relatively large size (mean size at day 0 = 27.6 mm) but that tadpoles in other Hs treatments, which were matched in starting size with corresponding Ls treatments, took considerably longer to reach any stage than those in the Ls treatments. The interactions were not significant (Table 1).

INCREMENT IN SIZE AT DAY 42
As with size at day 42, the data on size increment show a marked difference between sets of replicates, with the early replicates showing a far greater increment in size than the later replicates (effect of season: \( F_{1, 52} = 21.8, P < 0.001 \), Fig. 5). Growth increment was also suppressed by the presence of larger competitors with cohort once again having a highly significant effect (effect of cohort: \( F_{1, 52} = 9.3, P < 0.001 \)). Density, however, had a non-significant effect on size increment (\( F_{1, 52} = 2.2, P > 0.05 \)). There were no significant interactions (Table 1).

DISCUSSION
Competition clearly has adverse effects on *A. muletensis* larvae. Tadpoles suffer reduced growth and development, and the variation in their sizes increases, when they have to compete with large overwintered tadpoles. This may have arisen because the larger tadpoles monopolised most of the food and the hatchlings varied in their ability to compete for the remaining food. This, however, seems unlikely because food level was de- signed to be *ad libitum*. Therefore, the release of growth inhibitors (Richards, 1958; 1962; Beebee & Wong, 1992; Beebee, 1995; Griffiths, 1995; Petranka, 1995) or other interference competition (Akin, 1966; Steinwascher, 1979; Alford, 1998; Faragher & Jaeger, 1998), by the large larvae over the small larvae, cannot be ruled out.

In replicates 3 and 4 large tadpoles inhibited the growth of smaller tadpoles, but in replicates 1 and 2 there was little or no effect (Fig 5). This probably arises because the higher temperature in replicates 1 and 2 stimulated the tadpoles to start metamorphosing and, because these tadpoles were differentiating at a fast rate, little energy was being diverted to somatic growth. In replicates 3 and 4, however, tadpoles were not differentiating, and all available energy was being utilised for growth. Thus the relative effects of growth suppression were much higher in replicates 3 and 4 than in replicates 1 and 2. These results are consistent with models that state that growth and differentiation rates are independent, that resources are allocated to growth after they are allocated to differentiation, and that differentiation rate is fixed early in development (Smith-Gill & Berven, 1979; Leips & Travis, 1994).

The combination of large cohort and high density drastically retarded both growth and development in all the replicates. High density also had an important independent effect on reducing final size, but the non-significant effect on size increment and stage was probably due to the small sample size and the fact that the food ration was calculated on the clearance rate of the larger tadpoles. This meant that, even in the most competitive situations, there was sufficient food to allow some growth of even the smallest tadpoles. It is likely that density would have had a greater effect under conditions of higher food stress. Data on wild densities are few but two estimates are 20 and 59 tadpoles per m² (R. Griffiths pers. com.). However, nothing is known on food abundance in wild pools.

The influence of starting size was significant in determining final size but it had less of an effect than cohort, density or season. From this it seems likely that any advantages of being a large hatchling would only manifest themselves under conditions of low competitive stress. Thus, while hatching size is obviously important, the timing of tadpole deposition into the pools is probably more so. Being a large hatchling is advantageous, but not having to compete with overwintered tadpoles or high densities of tadpoles is more important in determining size and timing of metamorphosis.

The timing of breeding for prolonged breeders, especially with overwintering larvae, is very important because the nature of the competitive environment will change over time (Collins, 1979). *A. muletensis* breed from March-April to September a peak of activity in July-August (Bush, 1993), and metamorphic climax can take from a few days to two weeks depending on temperature (Lea, laboratory pers. obs). Males brood the eggs and carry them to water when the tadpoles are
ready to hatch. The low temperatures early in the season will lengthen the brooding period (Bush, 1993) and eggs will only gradually be deposited into the pools. Tadpole density should be low at this time but larvae may have to compete with the (large) overwintered tadpoles from the previous season. Competitive release (Travis, 1984) will occur as overwintered tadpoles start to metamorphose in mid-late May and, although density is increasing, hatchlings at this time should develop at a faster rate than earlier ones because of increasing temperature. By mid-June or July breeding is rapid and tadpole density will be high, there will also be some large overwintered tadpoles left that have not yet metamorphosed (Bush, 1993; Schley, 1996); competition at this time should be at its most intense. Late July–early August sees the rapid metamorphosis of large numbers of tadpoles, and whilst tadpoles are still being deposited, the overall density probably starts to decline. This again is a point of competitive release, and hatchlings should fare a little better. By September water temperature has dropped below 18°C (Bush, 1993 - no data on variation between pools and years), and below this temperature in the laboratory metamorphosis ceased (replicates 3 and 4), so tadpoles are perhaps now forced to overwinter.

In the field, a reduction in size, or lengthened larval period, may well reduce larval survival because of the increasing cumulative risks of predation (e.g. Heyer, McDiarmid & Weigman, 1975; Brodie & Formanowicz, 1983; Travis, Keen & Julianna, 1985), or being ‘washed out’ of the pools (Schley, 1996). Consequently, avoiding high densities and overwintering tadpoles should be advantageous. Also, breeding late in the season and prolonging the larval period may reduce lifetime reproductive success because reaching sexual maturity is dependent on a minimum age after metamorphosis in A. muletensis (Bush, 1993), and overwintered tadpoles will therefore breed one year later than hatchlings that do not overwinter. However, overwintered larvae would become large metamorphs and so larger, more fecund and perhaps more viable adults. Whether there is a size advantage to overwintering depends upon the relative growth rates in the aquatic and terrestrial environments (Werner, 1986). Similar trade-offs to the timing of breeding exist in Salamandra salamandra, where early or late cohorts are more successful depending on the amount of rainfall (Warburg, 1992; Griffiths, 1997).

The arguments above suggest that there should be selection to favour breeding during periods of competitive release (either side of mid-season) and perhaps avoid breeding at the end of the season, when hatchlings must overwinter in water and miss out on one year’s potential reproduction. However, the iteroparous nature of the females (within a season) and the limited availability of sexually receptive males (Bush 1993) may prolong the reproductive period, such that toads breed at optimal and sub-optimal times. Also, asynchronous breeding, and variation in competitive ability, means that tadpoles metamorphose over an extended period, which reduces the risk that a catastrophic event (e.g. flooding) will decimate the entire population (Griffiths, 1997).

Aquatic competition could affect population structure in the field by suppressing the growth of some larvae who would then possibly be smaller at first reproduction, and thus produce smaller hatchlings (Bush, 1993), which in turn may fare poorly under conditions of high competition or predation in the pools. Intense larval competition could also reduce larval or juvenile survival, and hence adult recruitment. Bearing in mind that A. muletensis is endangered; knowledge of the nature of larval competition can aid management strategies. For instance, the best times to introduce translocated tadpoles into new pools may be either side of mid-season during the periods of competitive release. Further field work needs to determine the relative predation pressures in the aquatic and terrestrial environments; how predator density and food availability changes across the season; and the relative contributions of larval and juvenile growth to adult size. Only with this information can we hope to determine what the optimal strategies are, for individuals seeking to maximise their fitness.

ETHICAL NOTE
Tadpoles used in the experiments came from a captive breeding and release programme that forms a component of the recovery programme for A. muletensis. No animal was deliberately harmed or killed for the purposes of this experiment.

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