A preliminary study on the effect of isolation on frog larval growth and metamorphosis

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ABSTRACT

Many tadpole species aggregate in nature and if larval densities are not too high there is generally a positive effect of grouping on growth and development. The benefits of grouping may be due to social facilitation and the availability of other tadpoles' faeces for consumption. We determined the effect of raising striped marsh frog (*Limnodynastes peronii*) tadpoles in isolation by comparing larval growth, size at metamorphosis and jumping distance at metamorphosis between full siblings raised in isolation, at low density and at high density. As expected, tadpoles raised in isolation experienced slower growth and development, which resulted in a prolonged larval duration and reduced mass and jump distance at metamorphosis. These results suggest that conspecific interactions are important for healthy growth and development of striped marsh frog larvae.

Key words: tadpole, *Limnodynastes peronii*, density effects, rearing conditions

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Introduction

Frog larvae, hereafter referred to as tadpoles, have been used extensively as model organisms for testing the effects of conspecific interactions on life history parameters. Tadpoles raised at high densities experience negative effects on growth and development such as a prolonged larval period, decreased size at metamorphosis and reduced growth rates (Gromko et al. 1973; Wilbur 1977; Dash and Hota 1980; Martinez et al. 1996). Previous work suggests that growth inhibitors from large tadpoles act as an interference mechanism and retard the growth of small tadpoles (Richards 1958; Rose 1960). A Prototheican unicellular alga was identified as a causative agent of interference growth inhibition in frog tadpoles and is transmitted by the consumption of faeces (coprophagy) (Beebee 1991; Beebee and Wong 1992). Competitive interference may also occur in a more direct manner by the vigorous swimming movements of large tadpoles that block the access of small tadpoles to food and prevents them from feeding efficiently (Wilbur 1977).

Although the negative effects of competition may be experienced at high densities, tadpole aggregation at lower densities has been shown to be beneficial due to the availability of faeces for coprophagy (Steinwascher 1978) and enhanced feeding by social facilitation (Gromko et al. 1973). The ingestion of faeces is a behavioural adaptation that increases the time food is present in the gut and promotes microbial digestion (Steinwascher 1978). When denied access to faeces by a mesh screen, tadpoles were smaller than treatments with full access to group faeces (Gromko et al. 1973; Steinwascher 1978). Social facilitation may enhance feeding as the movements of the group break food items into small pieces that are more readily available for consumption (Beiswenger 1975; Breden and Kelly 1982). Overall, a generally positive effect of social interaction is observed in tadpoles that normally aggregate in nature if the rearing density is not too high (Breden and Kelly 1982).

Raising tadpoles in isolation eliminates the interactions that may be necessary for healthy tadpole growth and development. Isolated tadpoles are not exposed to water-borne tadpole products from conspecifics such as faeces. Furthermore, isolated tadpoles cannot interact with each other, but in nature, tadpoles are rarely if ever found in water bodies without siblings with which they can interact (Anstis 2013). The aim of this study was to determine the effect of raising tadpoles in isolation on tadpole growth, development, mass at metamorphosis, larval duration and metamorph maximum hop distance. This was achieved by raising sibling striped marsh frog *Limnodynastes peronii* tadpoles in isolation and in low and high density groups and comparing the measured variables across treatments. We hypothesised that the benefits of social facilitation will outweigh the costs of competition and so tadpoles raised in isolation will experience slower growth and development rates and will attain a smaller size at metamorphosis than tadpoles raised together.

Methods

Collection and maintenance of tadpoles

A single *Limnodynastes peronii* egg mass was collected from a suburban pond on the Redcliffe peninsula, Queensland (27°13'37"S, 153°06'54"E) on the morning of 27 February 2008. Eggs were maintained at 25°C in pond water until hatching on 29 February 2008. Larvae were randomly allocated to one of three treatments: (Isolation (n=30), low density (n=30) and high density (n=60)). The low and high density treatments had only one replicate that

consisted of either 5 or 10 larvae.litre^{-1} respectively in 6 L of aged tap water in plastic containers (34×24×14 cm; L×W×H). The isolation treatment consisted of individual tadpoles in 0.2 L of water in round plastic containers (14×8 cm; D×H). Larvae were maintained at approximately 25°C and fed boiled lettuce leaves ad libidum. Water was replaced with aged, aerated tap water twice weekly.

Monitoring tadpole growth and development

Tadpoles were digitally imaged weekly from day 6 (5/03/2008). Tadpoles were staged weekly from day 28 (27/03/2008) using Gosner staging guidelines as modified by Anstis (2013) for Australian tadpoles. Tadpoles were individually removed from experimental treatments and placed in a shallow dish of water placed on top of gridded paper. Digital images were taken from above, and body width and length measurements made on images displayed on a computer screen (Fig. 1).

Upon emergence of forelimbs (Gosner stage 42) metamorphs were placed in individual containers with a small amount of water to complete metamorphosis. After complete tail reabsorption (Gosner stage 46) jumping tests were performed by first dipping metamorphs into a diluted food dye solution and stimulating them to jump by touching the urostyle with a fine hair artist's brush in a room at 25°C. The first three jump distances were measured to 1 mm with a ruler, and the maximum jump used for analysis. Frogs were then euthanized by chilling followed by freezing and dry mass calculated after drying in an oven at 50°C for 24 hours.

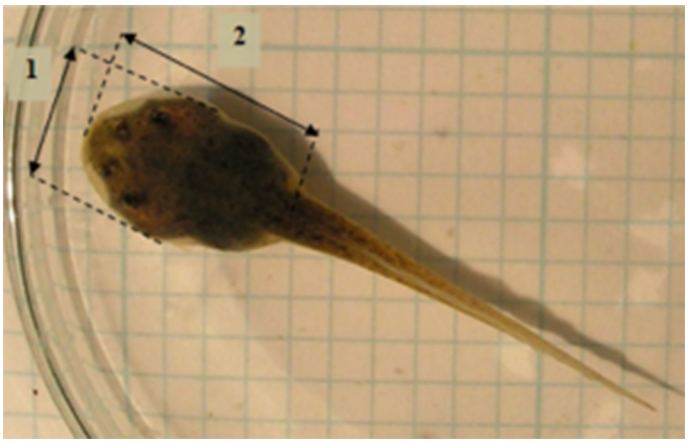

Figure 1. Photograph of *L. peronii* tadpole showing how body width (1) and length (2) were measured. Background grid paper was used to scale measurements to millimetres.

Data analysis

One-way ANOVA was used to test for differences in metamorph mass (mg), time to metamorphosis (days) and maximum jump distance at metamorphosis (mm). ANCOVA with metamorph dry weight as the covariate was used to correct for the effect of differences in metamorph size on maximum jump distance amongst rearing treatments. Tukey HSD for unequal sample sizes was used to make post hoc multiple comparisons between treatments. Because there was only one replicate of the low and high density treatments, statistical conclusions need to be interpreted cautiously. Statistica release 8 software was used for statistical analysis. To follow growth trajectories through time, body length (mm) and body width (mm) were multiplied to obtain a size index comparable across all tadpole treatments and this along with Gosner stage plotted against development time. Because from day 48 onwards tadpoles began to metamorphose and leave the water, from day 48 onwards the size data for each metamorphosed tadpole was carried through to consequent measurement days. Hence, after day 48, size data asymptote towards the mean size of all tadpoles in that treatment group at metamorphosis. Gosner stage data was treated in the same way, so after day 48, Gosner stage data asymptotes towards stage 46.

Results

Eight tadpoles from the isolated treatment, 8 from the low density treatment, and 10 from the high density treatment died before they reached metamorphosis leaving sample sizes of 22, 22, and 50 for the isolated, low density and high density treatments respectively. Some metamorphs died just after metamorphosis, which reduced the sample sizes for jumping tests to 15, 17 and 41 for isolation, low and high density treatments respectively.

Tadpole growth and development

Tadpole growth in all treatments was similar for the first 3 weeks (Fig. 2). After this time low density individuals were larger than both high density and isolated tadpoles. Metamorphosis was first observed in all treatments on day

Table 1. The effect of isolation on development and performance of *L. peronii* tadpoles (mean ± SE). ANCOVA with metamorph mass as the covariate was used to compare maximum jump distance adjusting for differences in metamorph mass. Least squares covariate means are reported.

Treatment	Dry mass at metamorphosis (mg)	Time to metamorphosis (days)	Maximum jump distance of metamorph (mm)	Maximum jump distance of metamorph adjusted to a dry mass of 56.8 mg (mm)
Isolation	48. 5^{a} ± 2.2	59.0^{a} ± 2.4	171^{a} ± 9	185 ± 11
Low density	74.0^{b} ± 3.8	48.7^{b} ± 0.9	218^{b} ± 10	192 ± 12
High density	55.1^{a} ± 3.0	51. 5^{b} ± 0.9	185^{a} ± 8	191 ± 7
F-statistic	12.23	11.17	4.65	0.093
P-value	<0.0001	<0.0001	0.0127	0.911

a,b Different superscript letters indicate significant differences as determined by a Tukey post hoc multiple comparison test.

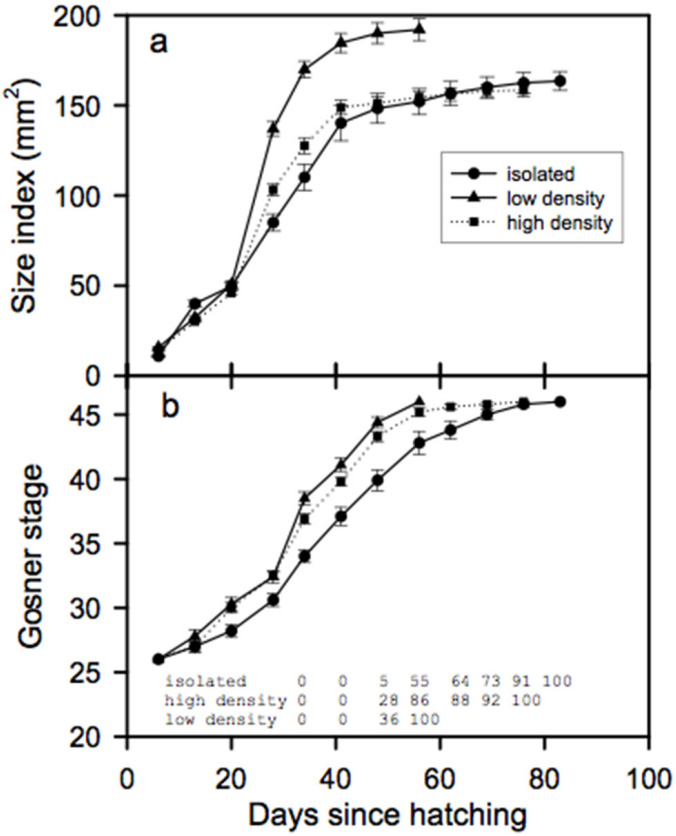

Figure 2. Plots of (a) size index and (b) Gosner stage with time after hatching of *L. peronii* tadpoles. Data points represent means and error bars represent standard errors. Numbers below lines in Gosner stage plots indicate the percent of tadpoles that had metamorphosed by that measurement day.

48, when one isolated, 8 low density and 14 high density tadpoles reached stage 46. All individuals metamorphosed by day 56 in the low density treatment, 76 d in the high density treatment and 86 d in the isolated treatment (Fig. 2). Isolated tadpoles took approximately 9 days longer to develop than tadpoles at low and high densities, and the variation in time to metamorphosis was much greater in isolated tadpoles (Table 1).

Tadpole metamorphs

Rearing treatment of tadpoles significantly affected mass at metamorphosis, time to metamorphosis and maximum jump distance of metamorphs (Table 1). Low density tadpoles were significantly heavier than isolated and high density tadpoles at metamorphosis, but isolated and high density metamorphs had similar mass (Table 1). Low density metamorphs jumped further than isolated and high density tadpoles (Table 1). Maximum jump distance was correlated with metamorph mass (Fig. 3) and once differences in body mass were accounted for by ANCOVA in which metamorph dry mass was the covariate, rearing treatment had no effect on maximum jump distance (Table 1).

Discussion

Limnodynastes peronii tadpoles raised in isolation and high density experienced overall negative effects on growth and development compared to tadpoles reared at low density, being smaller in mass and jumping shorter

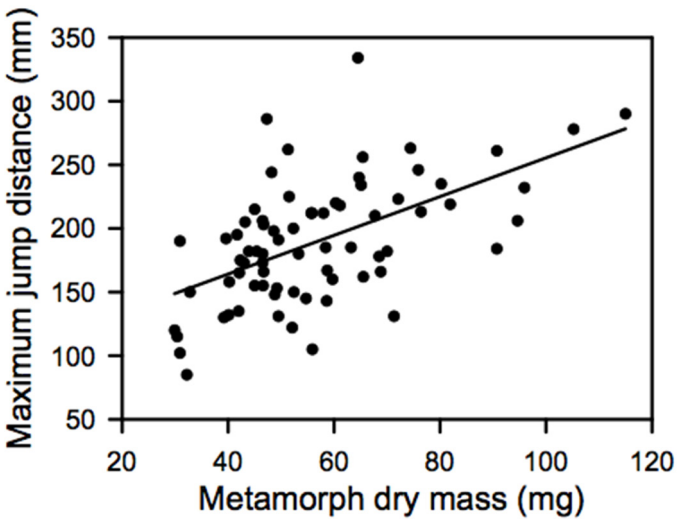

Figure 3. Correlation between metamorph dry mass and maximum jump distance. Regression line: $y = 103.3 + 1.52x$, $r^2 = 0.31$, $P < 0.001$, $N = 73$.

distance at metamorphosis. The shorter hopping distances were a direct result of the metamorphs being smaller in size as indicated by ANCOVA analysis and the direct relationship between maximum jump distance and dry mass. The adverse effects of high density rearing have been well documented for frog larvae, and have been attributed to physical interference disrupting feeding efficiency and growth inhibitors being released into water (Gromko et al. 1973; Wilbur 1977; Dash and Hota 1980; Beebee 1991; Beebee and Wong 1992; Martinez et al. 1996). However, the disadvantage caused by rearing in isolation has not been well document and is likely due to the lack of social facilitation, and the inability to ingest the faeces of siblings. Previous work on growth of frog tadpoles has shown that in group situations, the consumption of faeces may either enhance growth by providing an easily accessible food source (Gromko et al. 1973; Steinwascher 1978) or inhibit growth due to the effects of the parasitic unicellular Protothecan alga present in faeces (Beebee 1991). Tadpoles with access to faeces were shown to be larger than conspecifics that were prevented from feeding on faeces by screens (Gromko et al. 1973). In the high and low density treatments in this study, facilitated feeding may have occurred as many individuals fed simultaneously and enhanced food acquisition by the breaking apart of large lettuce pieces into smaller particles (Breden and Kelly 1982). Isolated tadpoles however were forced to independently manipulate their food portion and did not have the option of pre-digested faeces from other individuals which provide an easy to digest, microbe-enriched source of energy (Steinwascher 1978).

Amphibian metamorphosis has been modelled as a function of tadpole size and growth rate and can be initiated after a minimum threshold size is achieved (Wilbur and Collins 1973). Tadpoles reared in high density treatments experience slower growth rates and take longer to attain the minimum threshold size than those reared in low densities resulting in a prolonged larval period and a shift in the timing of metamorphic climax (Dash and Hota 1980). Tadpoles experiencing

high growth rates reach the lower threshold size earlier but may delay metamorphosis until they are larger in order to exploit the favourable conditions of the larval habitat (Wilbur and Collins 1973). If growth rate is slow, metamorphosis may be initiated immediately at the lower metamorphic size threshold due to the risk of predation or the onset of unfavourable conditions, such as pond desiccation (Wilbur and Collins 1973).

Tadpoles raised in isolation took approximately 9 days longer to metamorphose than tadpoles in the low and high density treatments. This suggests that isolated tadpoles experienced slow growth rates and upon reaching the threshold size immediately initiated metamorphosis. Tadpoles in the high density treatment had a shorter larval period than isolated tadpoles but metamorphosed at a similar mass. The mean dry mass at metamorphosis of tadpoles in the isolation treatment (49 mg) and high density treatment (55 mg) may reflect the lower size limit of metamorphosis for *L. peronii*. Tadpoles in the low density treatment had significantly larger average dry mass (74 mg) than both isolated and high density treatments. This suggests that these tadpoles experienced faster growth rates, possibly due to the benefits of grouping at low densities such as facilitation and coprophagy, and less intraspecific competition than in the high density treatment. As these tadpoles experienced favourable conditions they may have delayed the onset of metamorphosis until reaching a larger size.

The prolonged development time, smaller size and shorter maximum jump distance observed in tadpoles raised in isolation and at high density may have important implications for tadpoles in nature. Individuals that spend more time as tadpoles are more susceptible to death due to pond desiccation or predation by aquatic predators. As these animals are also smaller and have inferior jump performance at metamorphosis, they may also be susceptible to predation as metamorphs. Tadpoles have been raised in isolation as controls in studies of the effect of density on growth rate and metamorphosis (Dash and Hota 1980; Wilbur 1977). Such studies used the results from the isolation individuals as the null hypothesis of 'no effect' of density. The results of the current study suggest that using data from tadpoles raised in isolation as the 'growth trajectory' null model may not be valid as isolation has a negative, not a neutral, effect on tadpole growth. The decreased mass and maximum jump distance of isolated tadpoles was similar to that observed in high density individuals.

The difference in area available for swimming between the isolation and group treatments is a limitation of this study that may have contributed to the slower growth of isolated tadpoles. It has been shown that total area available for swimming may influence tadpole growth rates more than the volume of water per individual tadpole (John and Fenster 1975). As growth rates and mass at metamorphosis is proportional to container size for tadpoles raised in isolation (Rose 1960), the size of the container and volume of water may have been limiting growth in this experiment. In order to test whether the detrimental effects of isolation are due to the absence of conspecific interactions, or entirely due to container size, it would be necessary to raise tadpoles in isolation in varying quantities of water. Similar negative effects of isolation on tadpoles across different volumes of water would suggest that the isolation phenomenon occurs regardless of container size.

A previous study documented how exposure to varying levels of UV light affects *L. peronii* tadpole growth and metamorphosis (van Uitregt et al. 2007). In that study, as in others studies designed to examine variation in physical environmental variables on tadpole growth and development, experimental tadpoles were raised in isolation in individual containers so that individuals could be followed through time. However, in nature, tadpoles live in groups and are unlikely to be permanently isolated from their siblings. Given that our results indicate that rearing tadpoles in isolation adversely affects their growth rate, and size at metamorphosis, extrapolation of results from experiments where tadpoles were raised in isolation to the natural environment should be made with caution.

Acknowledgements

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