Correspondence

Crowding effects on growth rate of *Lithobates sphenocephalus* tadpoles (Amphibia: Anura: Ranidae)

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Numerous studies have demonstrated reduced growth of tadpoles with increasing density (reviewed in Wells 2007), and invoked factors such as food limitation or the accumulation of chemicals as possible mechanisms for such growth reductions. Some studies have explored the potential role of habitat space or water volume in driving these density effects. In most cases increased absolute water volume leads to higher growth rates (e.g., *Lithobates clamitans*, Pearman 1993; *Rhinella humboldti*, Montealegre-Delgado et al. 2013; *Engystomops pustulosus*, Montealegre-Delgado et al. 2013), whereas other species show reduced growth with absolute water volume (e.g., *Anaxyrus americanus*, Pearman 1993, *Argenteohyla siemersi pedersoni*, Kehr et al. 2014). Similarly, water depth and available surface area can also affect the growth of tadpoles, particularly those species whose tadpoles lack lungs (Calich & Wassersug 2012).

In addition, when the relative volume of water available for swimming (i.e., effective volume), as opposed to the absolute volume of water, decreases, the growth and development rate often decreases (Gromko et al. 1973, Golay & Durrer 1994, Smith 1998, Durnin & Smith 2001). One mechanism for these volume effects is an increase in the physical interactions between individuals as swimming volume decreases, and such interactions may lead to lower growth or development rates (John & Fenster 1975, John & Fusaro 1981, Rot-Nikcevic et al. 2005).

In this experiment, we specifically tested whether swimming area available to *L. sphenocephalus* tadpoles affected their growth. To this end, we maintained constant depth and volume of water, initial tadpole number, and food ration while manipulating the volume of water available to the tadpoles (i.e., swimming volume or effective volume). We predicted that tadpoles in the smallest swimming area would show the lowest growth rates due to increased physical interactions with other tadpoles.

We obtained eggs of *L. sphenocephalus* tadpoles from a commercial supplier (Charles D. Sullivan Co., Inc., Nashville, TN). We placed five tadpoles in circular plastic containers (17.3 cm diameter, 8 cm tall) filled with 1.2 l of aged tapwater. Experiments began 5 days after tadpoles had hatched when they were Gosner Stage 25 (Gosner 1960) and had a mean mass of 0.023 ± 0.001 g (N = 10 arbitrarily selected tadpoles). Using mesh enclosures constructed from window screening (1 mm mesh) and needlepoint frames (see Fig. 1), we created three effective volume treatments: small (7 cm diameter, 0.196 l), medium (9.3 cm diameter, 0.347 l), and large (no enclosure, 1.2 l), each replicated 5 times. We placed tadpoles inside the enclosures. Thus, all tadpoles were in the same absolute volume of water, but different effective volumes. The mesh enclosures were placed in the center of the containers and prior to construction, the needlepoint rings and mesh were soaked and aged in tap water for 7 days.

We fed tadpoles ground Purina Rabbit Chow twice every week (0.005 g per individual). Prior to feeding, we removed feces and excess food, and replaced any evaporated water in
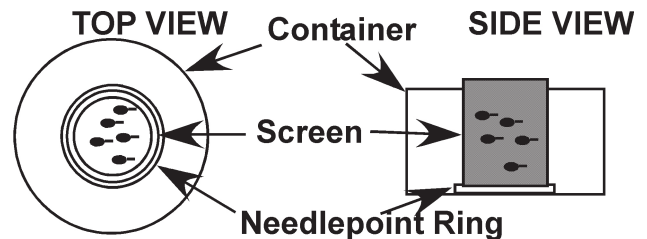

Figure 1. Diagram of experimental containers showing the basic design of the enclosures for the small and medium effective volume treatments. Large effective volume treatment lacked the screen enclosure. Diagram not drawn to scale.

each container. Every week we removed and replaced half of the water in each container. Water in the experimental containers was not aerated during the course of the study.

We ran the experiment for 17 days. There was no mortality in any treatment during the experiment. At the end of the experiment, we measured body length to the nearest 0.1 mm using a digital caliper, and weighed tadpoles to the nearest 0.001 g using an electronic balance after blotting the tadpoles dry. We used a MANOVA to compare body length, body mass, and body condition (BM/SVL) among

the effective volume treatments. We followed up the significant MANOVA with univariate ANOVAs. We used Tukey's HSD post-hoc tests to compare means of treatments for significant ANOVAs.

In the MANOVA, there was a significant effect of the effective volume of water available to the tadpoles (Wilks' λ = 0.28; $F_{4,22}$ = 4.86, p = 0.0058). Given the significant MANOVA, we ran univariate ANOVAs on tadpole length, mass, and condition.

Tadpoles in the large effective volume treatment were significantly longer than those grown in the other treatments (Fig. 2A; $F_{2,12}$ = 15.16, p = 0.0005). Tadpole mass was also affected by effective volume treatment, with tadpole mass increasing as swimming area increased (Fig. 2B; $F_{2,12}$ = 6.55, p = 0.012). However, tadpole body condition (BM/SVL) did not differ among the three effective volume treatments (Fig. 2C; $F_{2,12}$ = 1.56, p = 0.25).

By maintaining similar environmental conditions (the depth, absolute water volume, food, and chemical concentration) in each of the three effective volume treatments, we were able to test whether there was a direct effect on tadpole growth characteristics due to the physical area available for swimming. Our results support the conclusion that reductions in effective volume decrease the growth of *L. sphenocephalus* tadpoles suggesting tadpoles with more room for swimming grew more during our experiment. Our results are therefore consistent with previous experiments that have found that tadpole growth is negatively affected by smaller effective or swimming volume (Gromko et al. 1973, Golay & Durrer 1994, Smith 1998, Durnin & Smith 2001). Because all tadpoles in our experiment experienced the same food level and absolute water volume, these results strongly suggest that so-called density effects can arise independent of food limitation or chemical accumulation. In particular, the use of screening probably allowed chemical wastes produced by the tadpoles to disperse out of the enclosed area, and the frequency and extent of water changes we used likely reduced the accumulation of any chemical wastes. Our results therefore lend credence to the idea that the increased incidence of physical interactions among individuals is partially responsible for such effects (see John & Fenster 1975, John & Fusaro 1981, Rot-Nikcevic et al. 2005). It is also possible that tadpoles in the different effective volume treatments differed in activity or other behavior which might affect their growth. We did not make observations of tadpole behavior that could address this possibility. Additional experiments that simultaneously manipulate effective volume and food availability would be able to better elucidate the relative importance of physical interactions and food limitation in tadpole density effects.
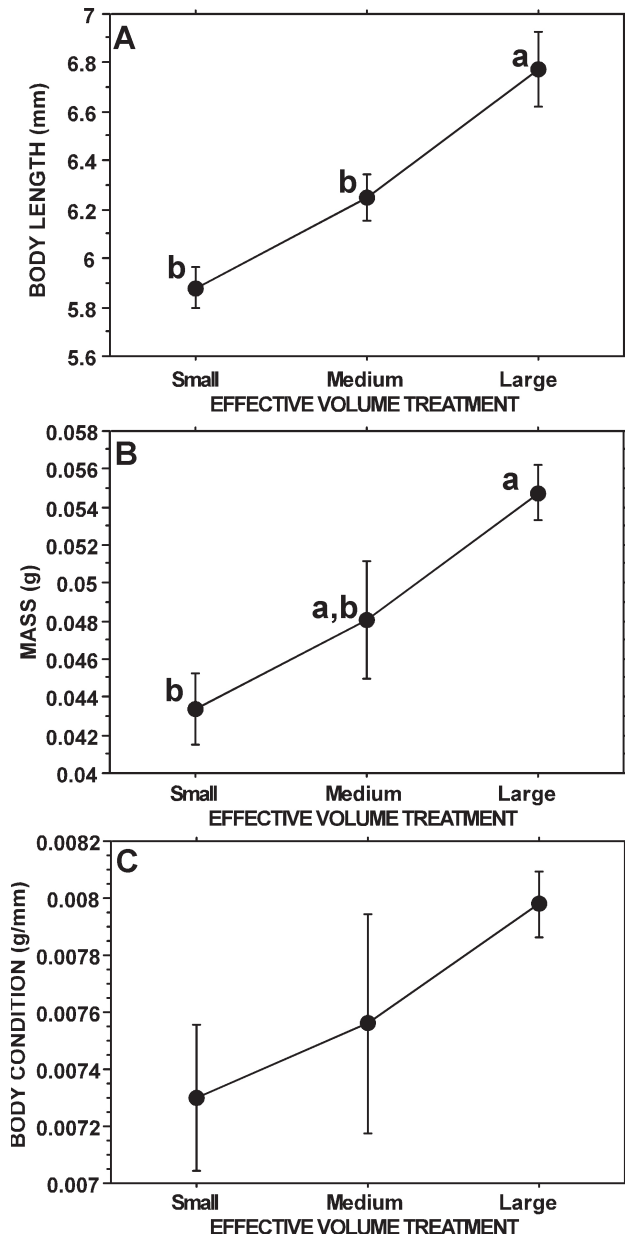

Figure 2. The effects of effective volume treatments on (A) mean body length, (B) mean tadpole mass, and (C) mean body condition of *Lithobates sphenocephalus*. Means are given ± 1 SE. Means sharing the same letter are not significantly different (Tukey HSD post-hoc tests).

Acknowledgments

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