Food, not friend: Tadpoles of the sandpaper frog (*Lechriodus fletcheri*) cannibalise conspecific eggs as a food resource in ephemeral pools

Running title: Cannibalism in *Lechriodus fletcheri*

John Gould[1], John Clulow[1] & Simon Clulow[1,2]

[1]School of Environmental and Life Sciences, University of Newcastle, Callaghan, 2308 NSW, Australia

[2] Department of Biological Sciences, Macquarie University, Sydney, 2109 NSW, Australia

Acknowledgements

The authors thank Jose Valdez for his guidance during this study. This work was conducted under NPWS Scientific license (no. SL101991) and approved by the University of Newcastle Animal Care and Ethics Committee (no. A-2011-138). All experimental procedures were performed in accordance with the Australian code for the care and use of animals for scientific purposes.

Correspondence

John Gould, Conservation Biology Research Group, School of Environmental and Life Sciences, University of Newcastle, University Drive, Callaghan, NSW 2308, Australia

Email: John.Gould@uon.edu.au

Conflict of interest

The authors wish to state that there are no sources of conflict of interest.

Abstract

Among the Amphibia, cannibalism is most commonly associated with tadpole species that exploit ephemeral systems. This behaviour may confer significant fitness benefits to those that cannibalise, given that these systems generally possess limited food resources, but will incur significant fitness costs to the cannibalised. Herein, we describe cannibalism of recently oviposited eggs of the sandpaper frog (*Lechriodus fletcheri*) by conspecific tadpoles as a likely adaptation to limited nutrient availability within highly ephemeral pools in which it is an obligate breeder. Field observations revealed *L. fletcheri* tadpoles actively preyed on conspecific eggs of recently oviposited spawn bodies, which were commonly consumed whole. When tadpoles were exposed to spawn for the first time in laboratory trials, they quickly engaged in extended periods of consumption, gorging themselves until they appeared to be full. We found this behaviour to be common in the field and suggest that conspecific eggs form a significant food resource for tadpoles of this species in the otherwise nutrient poor systems in which they breed. This feeding strategy might be common among anurans exploiting temporary aquatic systems that are nutrient-poor and gives rise to many questions surrounding how individuals can utilise cannibalism to increase their fitness while simultaneously avoiding becoming victims of this behaviour themselves.

Keywords: amphibia, anuran, cannibalism, feeding strategy, intraspecific predation, nutrient acquisition

Introduction

As most amphibian species are oviparous and provide limited parental care (Gross & Shine, 1981), their offspring are particularly vulnerable to predation while they are still completing embryogenesis, given that they are immobile prior to hatching and cannot evade this threat even if detected (Chivers et al., 2001; Touchon, Gomez-Mestre, & Warkentin, 2006). It is no surprise then that amphibian embryos are consumed by a variety of natural aquatic predators (Karraker & Dudgeon, 2014; Monello & Wright, 2001; Reshetnikov, 2003), including other amphibian species (Kats, Breeding, Hanson, & Smith, 1994; Walters, 1975). Under some circumstances, they may also be vulnerable to predation by conspecifics via forms of cannibalism.

Cannibalism has been documented among amphibians at several life history stages (Polis & Myers, 1985), including the active predation of smaller conspecifics by adults and the opportunistic cannibalism of dead or dying tadpoles or active predation of healthy tadpoles by conspecific at the same life-history stage. The consumption of eggs by conspecifics has also been recorded and is a significant source of mortality for some species (Crump, 1983; Polis & Myers, 1985). Like other forms of cannibalism, it is more common in systems where there is a high density of individuals present in conjunction with limited food resources (Collins & Cheek, 1983; Semlitsch & Reichling, 1989; Walls, 1998). Under these conditions, feeding on conspecific eggs may confer significant fitness benefits for offspring not only in the form of acquired nutrition for enhanced growth, but also through reductions in future competition for resources (Crump, 1983, 1990; Nagai, Nagai, & Nishikawa, 1971).

Most amphibian offspring are only provisioned nutrients in the form of yolk to complete embryogenesis, with very few species known to provide additional nutrients to offspring after hatching through forms of parental care (Weygoldt, 1980). Cannibalism could be an effective strategy for tadpoles to sustain their continued development post-hatching. Yet, this comes at a cost by necessity to others in the population. In order to increase fitness through cannibalism, reproducing adults must enable their offspring the opportunity to take advantage of cannibalism without becoming victims themselves. As such, complex questions arise as to the choices of adults when it comes to oviposition site selection and reproductive timing, as this will dictate whether offspring will become cannibals or cannibalised. Answering such questions, however, is often hampered by a lack of basic understanding or documentation of the level of cannibalism that might occur in any particular species, and the degree to which a species might be reliant on this strategy for nutrients in resource-limited environments.

Ephemeral waterbodies are ideal settings for the evolution of cannibalism, given that food availability in these systems may be unpredictably variable and often limited as a result of their tendency to dry out. Considering the timing of water inundation, amphibians that utilise these systems usually engage in explosive breeding, synchronising the laying of their spawn with the onset of rainfall to maximise the availability of water during offspring development (Petranka & Thomas, 1995; Wells, 1977). While such aggregations will lead to increased food competition for offspring upon hatching, it also will result in the temporary influx of high concentrations of conspecific eggs during each breeding event (Audo et al., 1995; Crump, 1983). Previously hatched tadpoles that are able to exploit these eggs as a transient nutrient supply will be at a great advantage, particularly as they race to reach metamorphosis before their pools inevitably dry out. To date, the consumption of conspecific eggs by tadpoles has been recorded in several amphibian species, most noticeably arboreal breeders that supply unfertilised eggs to their young in small pools that form in tree holes and bromeliads (Noble & Pope, 1929; Taylor, 1954; Weygoldt, 1980). Herein, we provide the first data on the cannibalisation of eggs by tadpoles of the sandpaper frog (*Lechriodus fletcheri*), as a likely adaptation to poor nutrient availability within the highly ephemeral pools of which it is an obligate breeder (Clulow & Swan, 2018). We aimed to document this behaviour to support future studies on how amphibians might use cannibalism to adapt to reproducing in nutrient-poor ephemeral aquatic systems, without becoming victims themselves.

Materials and Methods

Study species:

Lechriodus fletcheri is a medium size anuran (4 to 5 cm) that occupies temperate forests along the Australian east coast (Clulow & Swan, 2018). Although breeding occurs across an extended season (September through till March), it is confined to periods of heavy rainfall, given that the highly ephemeral pools it uses for egg deposition often dry out within a matter of days or weeks after rainfall has ceased. Amplecting pairs produce a foamy mass of approximately 300 fertilised eggs that remains floating on the surface of the water while the offspring complete embryogenesis. Development in this species is rapid, with hatching of free swimming tadpoles occurring after just 3 to 4 days, and metamorphosis occurring in as little as 20 to 30 days (Anstis, 2017).

Cannibalism in the field:

Field observations for this study took place within the Watagan Mountain Range, New South Wales, Australia (33° 00' 30.6 S, 151° 23' 15.7 E) during field work on *L. fletcheri* for a companion study. We investigated whether cannibalism of conspecific eggs was prevalent in this population by examining 11 pools that were found to contain both recently oviposited spawn and older, previously hatched tadpoles. On October 12, 2017, the feeding behaviour of tadpoles within these pools was observed for a period of ten minutes each. As surveys were conducted at night, a head torch was used to illuminate the water column so that individuals could be easily seen. Records were made on whether tadpoles made contact with spawn present in their respective pools. When contact was made, we paid close attention to any signs of mouthpart movement to determine whether tadpoles were trying to consume the spawn. After observations had been made without interference, 20 tadpoles were collected from one of the pools via dip-netting in order to determine their developmental stage (Gosner, 1960) and to visually examine the contents of their stomachs post-feeding. Observations of the intestinal tract were made through the translucent skin of the underbelly to determine whether tadpoles had been feeding on just the frothed oviduct fluid of the spawn body or the residing embryos as well. Tadpoles were unharmed during this process and returned to their pool of origin within 30 seconds of capture.

Cannibalism in the laboratory:

To further investigate how *L. fletcheri* tadpoles feed upon conspecific spawn and eggs, and to examine whether tadpoles would commence cannibalistic behaviour in the presence of other food sources such as algae, we conducted a small laboratory experiment. Two recently oviposited spawns containing 100-150 eggs were collected from the field and brought back to the laboratory, where they were placed into separate 27 x 17 x 15 cm containers filled with aged tap water. Once the residing embryos hatched (approximately four days later), they were fed dried Spirulina powder *ad libitum*. Approximately 15 days later when tadpoles had reached Gosner stage 34 of development (Gosner, 1960), two newly oviposited spawn containing 100-150 eggs were collected from the field and placed into the containers. Two additional spawn containing a similar number of eggs were placed into containers that lacked tadpoles to act as controls and to account for natural attrition and decay in the absence of any predators. Spirulina was added to the containers 10 to 20 minutes prior to the introduction of spawn, though no additional Spirulina was added once spawn had been introduced. We recorded whether tadpoles, which up until that point had only been fed on algae, chose to feed on spawn by observing their feeding behaviour. Containers were visually monitored twice daily over ten minute intervals, which was repeated over two consecutive days. During observations, close attention was paid to the tadpole's mouthparts during feeding and possible changes in their size that would be suggestive of the consumption of large amounts of spawn material. Photographs and video recordings were taken during observational periods using an iPhone 6 (Apple Corporation, Cupertino, California, United States).

Results

Cannibalism in the field:

Lechriodus fletcheri tadpoles were observed feeding around the perimeter of newly oviposited conspecific spawn in each of the 11 pools that were surveyed. A small number of tadpoles (5 to 10) were also observed feeding from beneath each spawn, which was apparent by the movement of the tadpoles as they 'nudged' the underside of frothed oviduct fluid. All tadpoles present within these pools were of a similar stage of development (Gosner 31- 35), with no sympatric tadpole species observed. The stomach contents of tadpoles that were collected from one pool site were found to contain intact *L. fletcheri* eggs, with the distinct hemispheres of the early stage embryos clearly visible through the ventral skin. Approximately 1-3 intact eggs were present in the intestinal tracts of a majority of these tadpoles. One individual was found to have recently fed on at least eight eggs, with this large mass of eggs resulting in the tadpole possessing a distended abdomen (Fig. 1). In another one of these pools, an entire spawn had been completely devoured prior to the commencement of surveys, with only a small remanet of oviduct fluid left.

Cannibalism in the laboratory:

Tadpoles reared under laboratory conditions readily fed on *Spirulina* algae that were added to their containers. Feeding usually occurred immediately at the surface of the water where the algae was added, with tadpoles ingesting the material by repeatedly gulping at the water. Most tadpoles were found to quickly transition to feeding on freshly oviposited spawn once it was added to the containers. Similar to observations made in the field, the feeding behaviour of *L. fletcheri* tadpoles commonly involved the consumption of conspecific eggs located around the immediate perimeter of the spawn, although feeding also frequently occurred from the underside (Fig. 2). During feeding, each individual made contact with the exposed edge of the spawn and began to gulp on the closest section of frothed oviduct fluid that was exposed to the water (Fig. 3). Periodically, tadpoles would thrash from side-to side in short, whipping like motions to detach a small section of material before consuming it.

As with field observations, tadpoles kept in the lab were observed to consume whole eggs when they encountered them interspersed through the spawn (i.e. not just the surrounding jelly capsules). On some occasions, tadpoles would remove sections of oviduct fluid containing a small number of eggs, which were slowly consumed whole. Tadpoles would often remain near the spawn for extended periods of time and gorge themselves, with most showing signs of distended abdomens that were full of spawn material. It became apparent that eggs closest to the perimeter of the spawn were the first to be consumed, with those present deeper within the spawn body unable to be reached for consumption until a later point in time after surrounding material had been removed. After two days, all of the eggs from both containers containing tadpoles had been entirely consumed, including the surrounding oviduct fluid of the spawn body itself. Although some embryos were able to successfully hatch before this time, they too were cannibalised, both at the surface of the water and at the bottom of the container. All embryos from the control containers developed and hatched into tadpoles.

Discussion

We showed that *L. fletcheri* tadpoles readily consume conspecific spawn, feeding on the frothed oviduct fluid of the spawn body and cannibalising the residing eggs. The consumption of eggs was observed across all pools surveyed that contained recently oviposited spawn, suggesting that it is a common feeding behaviour for this species. It is likely that tadpoles at all stages of development pose a predatory risk to conspecific eggs, except for those that have only just recently hatched, as they will remain immobile at the surface of the water and unlikely to feed over the first 24-48 hours post-hatching. While these findings are similar to reports of some other anurans that breed in temporary pools (Crump, 1983; Petranka & Thomas, 1995), this is the first recorded case of tadpoles of an Australian ground frog (Limnodynastidae) consuming conspecific eggs.

We suggest that this form of cannibalism is likely to have evolved in *L. fletcheri* as a direct and deliberate response to low nutrient availability in the small and highly ephemeral breeding sites which it exploits. Limited food resources are particularly problematic for tadpoles that need to obtain sufficient nutrients to be able to develop and metamorphose quickly before desiccation occurs. In an environment that is otherwise food scarce, un-hatched conspecifics provide an easily obtainable and nutritious feed high in minerals, lipids and proteins (Crump, 1983), which may quicken development. There is also a high chance of this interaction between conspecific life history stages, given that this species undergoes ‘explosive’ breeding (Clulow & Swan, 2018), leading to breeding pools often containing older conspecific tadpoles by the time new spawn is oviposited, provided the pools have not already dried out. Such a hefty meal, though likely intermittent, allows tadpoles to increase in size and develop more rapidly (Nagai et al., 1971), which in turn confers fitness benefits (Leips & Travis, 1994). Consuming conspecifics before they’ve hatched also reduces future competition and predation threats for those offspring that are fortunate enough not to be cannibalised themselves before they have hatched (Ziemba & Collins, 1999) and reduces overcrowding, which can have potentially negative effects such as the depletion of oxygen availability and increased disease transmission (Polis, 1981).

Given how quickly tadpoles can devour conspecific spawn, it might be expected that adult pairs would attempt to avoid ovipositing in pools that already contain older tadpoles in order to prevent their offspring becoming victims of cannibalism. Oviposition site selection has previously been recorded in this species with adults selecting pools of an optimal temperature for tadpole development (Garnham, Mahony, & Clulow, 2012). It might be possible that site selection also occurs in relation to the presence of conspecific tadpoles (avoidance), as a mechanism to prevent offspring from becoming cannibalised. If so, selection should favour individuals that are the first to breed after rainfall has re-filled pools with water. However, no benefit will come from laying earlier versus later in the season given that a majority of pools will dry repeatedly. This regular disturbance, along with the rapid rate of tadpole development seen in *L. fletcheri*, will provide opportunities for spawning in tadpole-free pools throughout the season. Paradoxically, site selection in relation to fresh spawn (attraction) may also occur, particularly if the presence of spawn is a signifier of pool quality (Rudolf and Rödel, 2005). If a potential breeding site contains recently oviposited spawn but not previously hatched tadpoles, then there will be little risk of egg predation for any additional spawn deposited over this period, particularly as tadpoles remain immobile at the surface of the water over the first few days post-hatching. On the contrary, these neighbouring spawn may eventually be an important source of future nutrients, given that *L. fletcheri* tadpoles are also known to be cannibalistic towards conspecifics at the same life history stage (Martin 1967). It is therefore the timing of an adult's reproductive activity in relation to others in the population that will be critical in determining the fate of their offspring.

It appears that *L. fletcheri* eggs lack many of the anti-predator defence mechanisms identified in other tadpole species, such as being unpalatable or surrounded by a tough coating (Gunzburger & Travis, 2005; Ward & Sexton, 1981), meaning they will have no means of reducing their exposure to this threat. Although yet to be investigated, it is possible that the spawn body in which the eggs develop may serve as a physical barrier that provides some level of protection against predation. Producing a frothed spawn body is thought to reduce offspring predation risk, as well as desiccation threat in some species that exploit ephemeral aquatic systems (Hödl, 1990). As *L. fletcheri* eggs remain floating on the surface of the water in these masses throughout development, only those eggs closest to the bottom surface and perimeter of the spawn body will be exposed and easily cannibalised. In contrast, those eggs buried deep within the spawn will be provided more time to complete their development before they too become vulnerable to predation, as surrounding spawn will first need to be consumed before they are exposed.

Although the transition away from more permanent systems is generally considered to reduce the vulnerability of early developmental stages to aquatic predators (Duellman & Trueb, 1986; Heyer, McDiarmid, & Weigmann, 1975), these findings suggest that it may invariably increase the intensity of predation by conspecifics. While this may appear at first glance to be highly disadvantageous for the species, it is likely to improve the chance of survival of some individuals, albeit at the expense of their fellow conspecifics. This in turn gives rise to many interesting questions around how individuals reproduce in a way that affords their offspring an ability to take advantage of this behaviour without falling victims to it themselves.

References

Anstis, M. (2017). *Tadpoles and frogs of Australia*. New Holland Publishers Pty Limited.

Audo, M., Mann, T., Polk, T., Loudenslager, C., Diehl, W., & Altig, R. (1995). Food deprivation during different periods of tadpole (*Hyla chrysoscelis*) ontogeny affects metamorphic performance differently. *Oecologia, 103*(4), 518-522. doi:10.1007/BF00328691

Chivers, D. P., Kiesecker, J. M., Marco, A., Devito, J., Anderson, M. T., & Blaustein, A. R. (2001). Predator-induced life history changes in amphibians: egg predation induces hatching. *Oikos, 92*(1), 135-142. doi:10.1034/j.1600-0706.2001.920116.x

Clulow, S., & Swan, M. (2018). *A complete guide to frogs of Australia*: Australian Geographic.

Collins, J. P., & Cheek, J. E. (1983). Effect of food and density on development of typical and cannibalistic salamander larvae in *Ambystoma tigrinum nebulosum*. *American Zoologist, 23*(1), 77-84. doi:10.1093/icb/23.1.77

Crump, M. L. (1983). Opportunistic cannibalism by amphibian larvae in temporary aquatic environments. *The American Naturalist, 121*(2), 281-289. doi:10.1086/284058

Crump, M. L. (1990). Possible enhancement of growth in tadpoles through cannibalism. *Copeia, 1990*(2), 560-564. doi:10.2307/1446361

Duellman, W., & Trueb, L. (1986). Biology of Amphibians McGraw-Hill. *New York*.

Garnham, T., Mahony, M., & Clulow, S. (2012). *Overcoming unpredictability: investigating oviposition site selection in the sandpaper frog, Lechriodus fletcheri, as an adaptive strategy in the face of unpredictable change* (Honours thesis), The University of Newcastle, Australia.

Gosner, K. L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica, 16*(3), 183-190.

Gross, M. R., & Shine, R. (1981). Parental care and mode of fertilization in ectothermic vertebrates. *Evolution, 35*(4), 775-793. doi:10.2307/2408247

Gunzburger, M. S., & Travis, J. (2005). Critical literature review of the evidence for unpalatability of amphibian eggs and larvae. *Journal of Herpetology, 39*(4), 547-572. doi:10.1670/1-05A.1

Heyer, W. R., McDiarmid, R. W., & Weigmann, D. L. (1975). Tadpoles, predation and pond habitats in the tropics. *Biotropica*, 100-111. doi:10.2307/2989753

Hödl, W. (1990). An analysis of foam nest construction in the neotropical frog *Physalaemus ephippifer* (*Leptodactylidae*). *Copeia*, 547-554. doi:10.2307/1446358

Karraker, N. E., & Dudgeon, D. (2014). Invasive apple snails (*Pomacea canaliculata*) are predators of amphibians in South China. *Biological invasions, 16*(9), 1785-1789. doi:10.1007/s10530-014-0640-2

Kats, L. B., Breeding, J. A., Hanson, K. M., & Smith, P. (1994). Ontogenetic changes in California newts (*Taricha torosa*) in response to chemical cues from conspecific predators. *Journal of the North American Benthological Society, 13*(2), 321-325. doi:10.2307/1467250

Leips, J., & Travis, J. (1994). Metamorphic responses to changing food levels in two species of *hylid* frogs. *Ecology, 75*(5), 1345-1356. doi:10.2307/1937459

Martin, A. A. (1967). The biology of tadpoles. *Australian Natural History*, *15*(10), 326-330.

Monello, R. J., & Wright, R. G. (2001). Predation by goldfish (*Carassius auratus*) on eggs and larvae of the eastern long-toed salamander (*Ambystoma macrodactylum columbianum*). *Journal of Herpetology, 35*(2), 350-353. doi:10.2307/1566132

Nagai, Y., Nagai, S.-i., & Nishikawa, T. (1971). The nutritional efficiency of cannibalism and an artificial feed for the growth of tadpoles of Japanese Toad (*Bufo vulgaris* sp.). *Agricultural and Biological Chemistry, 35*(5), 697-703. doi:10.1080/00021369.1971.10859982

Noble, G. K., & Pope, C. H. (1929). The adaptive modifications of the arboreal tadpoles of *Hoplophryne* and the torrent tadpoles of *Staurois*. Bulletin of the AMNH; v. 58, article 7.

O'Neill, B. J., & Thorp, J. H. (2014). Untangling food-web structure in an ephemeral ecosystem. *Freshwater Biology, 59*(7), 1462-1473. 10.1111/fwb.12358

Petranka, J. W., & Thomas, D. A. (1995). Explosive breeding reduces egg and tadpole cannibalism in the wood frog, *Rana sylvatica*. *Animal Behaviour, 50*(3), 731-739.

Polis, G. A. (1981). The evolution and dynamics of intraspecific predation. *Annual Review of Ecology and Systematics, 12*(1), 225-251. doi:10.1146/annurev.es.12.110181.001301

Polis, G. A., & Myers, C. A. (1985). A survey of intraspecific predation among reptiles and amphibians. *Journal of Herpetology*, 99-107. doi:10.2307/1564425

Reshetnikov, A. N. (2003). The introduced fish, rotan (*Perccottus glenii*), depresses populations of aquatic animals (macroinvertebrates, amphibians, and a fish). *Hydrobiologia, 510*(1-3), 83-90. doi:10.1023/B:HYDR.0000008634.92659.b4

Rudolf, V. H., & Rödel, M-O. (2005). Oviposition site selection in a complex and variable environment: the role of habitat quality and conspecific cues. *Oecologia* 142, 316-325. doi: 10.1007/s00442-004-1668-2.

Semlitsch, R. D., & Reichling, S. B. (1989). Density-dependent injury in larval salamanders. *Oecologia, 81*(1), 100-103. doi:10.1007/BF00377017

Taylor, E. H. (1954). *Frog-egg Eating Tadpoles of Anotheca Coronata (Stejneger).(Salientia, Hylidae)*: University of Kansas.

Touchon, J., Gomez-Mestre, I., & Warkentin, K. (2006). Hatching plasticity in two temperate anurans: responses to a pathogen and predation cues. *Canadian Journal of Zoology, 84*(4), 556-563. doi:10.1139/z06-058

Walls, S. C. (1998). Density dependence in a larval salamander: the effects of interference and food limitation. *Copeia*, 926-935. doi:10.2307/1447339

Walters, B. (1975). Studies of interspecific predation within an amphibian community. *Journal of Herpetology*, 267-279. doi:10.2307/1563191

Ward, D., & Sexton, O. J. (1981). Anti-predator role of salamander egg membranes. *Copeia, 1981*(3), 724-726. doi:10.2307/1444586

Wells, K. D. (1977). The social behaviour of anuran amphibians. *Animal Behaviour, 25*, 666-693. doi:10.1016/0003-3472(77)90118-X

Weygoldt, P. (1980). Complex brood care and reproductive behaviour in captive poison-arrow frogs, *Dendrobates pumilio O*. Schmidt. *Behavioral ecology and sociobiology, 7*(4), 329-332. doi:10.1007/BF00300674

Ziemba, R. E., & Collins, J. P. (1999). Development of size structure in tiger salamanders: the role of intraspecific interference. *Oecologia, 120*(4), 524-529. doi:10.1007/s004420050886

Figures

Figure 1. *Lechriodus fletcheri* tadpole with numerous conspecific eggs that had been consumed whole clearly visible inside the gut. The tadpole was collected from a natural waterbody within the field study area after feeding on recently oviposited conspecific spawn.

Figure 2. *Lechriodus fletcheri* tadpoles feeding on the underside of a conspecific spawn that was recently laid. This feeding behaviour was less commonly observed than feeding from the perimeter of the spawn.

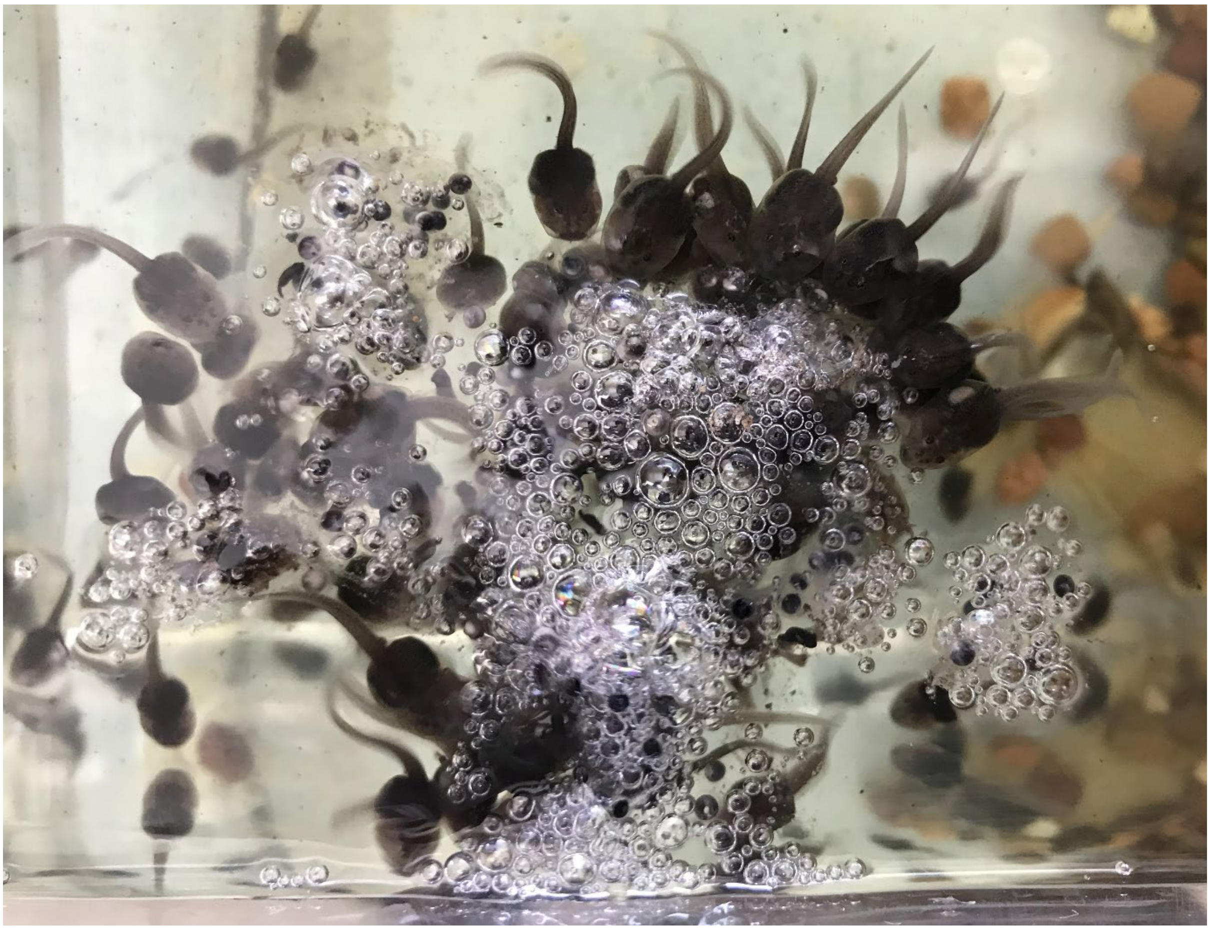

Figure 3. *Lechriodus fletcheri* tadpoles feeding around the perimeter of a conspecific spawn that was recently laid, as was mostly commonly observed. The spawn has already been nearly entirely consumed, with few eggs remaining.