

- of coral endosymbionts and coral tissue lesions. *PLoS ONE*, 2009, **4**, e4511; doi:10.1371/journal.pone.0004511.
20. Ben-Haim, Y. and Rosenberg, E., A novel *Vibrio* sp. pathogen of the coral *Pocillopora damicornis*. *Mar. Biol.*, 2002, **141**, 47–55.
 21. Meron, D. *et al.*, Role of flagella in virulence of the coral pathogen *Vibrio coralliilyticus*. *Appl. Environ. Microbiol.*, 2009, **75**, 5704–5707.
 22. Schmidt, A. S., Bruun, M. S., Dalsgaard, I., Pedersen, K. and Larsen, J. L., Occurrence of antimicrobial resistance in fish – pathogenic and environmental bacteria associated with four Danish rainbowtrout farms. *Appl. Environ. Microbiol.*, 2000, **66**, 4908–4915.
 23. Okoh, A. I. and Igbinsosa, E. O., Antibiotic susceptibility profiles of some *Vibrio* strains isolated from wastewater final effluents in a rural community of the Eastern Cape Province of South Africa, *BMC Microbiol.*, 2010, **10**, 143.
 24. Hua, L. M. and Apun, K., Antimicrobial susceptibilities of *Vibrio parahaemolyticus* isolates from tiger shrimps (*Penaeus monodon*) aquaculture in Kuching, Sarawak. *Res. J. Microbiol.*, 2013, **8**, 55–62.
 25. Hsu, C. H., Hwang, S. C. and Liu, J. K., Succession of bacterial drug resistance as an indicator of antibiotic application in aquaculture. *J. Fish. Soc. Taiwan*, 1992, **19**, 55–64.
 26. Manjusha, S., Sarita, G. B., Elyas, K. K. and Chandrasekaran, M., Multiple antibiotic resistances of *Vibrio* isolates from coastal and brackish water areas. *Am. J. Biochem. Biotechnol.*, 2005, **1**, 201–206.
 27. Molina-Aja, A., Garcia-Gasca, A., Abreu-Grobois, A., Bolan-Mejia, C., Roque, A. and Gomez-Gill, B., Plasmid profiling and antibiotic resistance of *Vibrio* strains isolated from cultured penaeid shrimp. *FEMS Microbiol. Lett.*, 2002, **213**, 7–12.
 28. Zhang, R., Wang, Y. and Gu, J. D., Identification of environmental plasmid-bearing *Vibrio* species isolated from polluted and pristine marine reserves of Hong Kong, and resistance to antibiotics and mercury. *Antonie van Leeuwenhoek*, 2006, **89**, 307–315.
 29. Guiney, D. G. and Landa, E., Conjugative transfer of Inc plasmids. In *Promiscuous Plasmids of Gram-negative Bacteria* (ed. Thomas, C. M.), Academic Press, London, 1989, pp. 27–56.
 30. Devi, R., Surendran, P. K. and Chakraborty, K., Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from shrimp farms along the southwest coast of India. *World J. Microbiol. Biotechnol.*, 2009, **25**, 2005–2012.
 31. Tiainen, T., Pedersen, K. and Larsen, J. L., Ribotyping and plasmid profiling of *Vibrio anguillarum* serovar O₂ and *Vibrio ordalii*. *J. Appl. Bacteriol.*, 1995, **79**, 384–392.
 32. Radu, S. *et al.*, Characterization of *Vibrio vulnificus* isolated from cockles (*Anadara granosa*): antimicrobial resistance, plasmid profiles and random amplification of polymorphic DNA analysis. *FEMS Microbiol. Lett.*, 1998, **165**, 139–143.
 33. Sorum, H., Hvaal, A. B., Heum, M., Daae, F. L. and Wiik, R., Plasmid profiling of *Vibrio salmonicida* for epidemiological studies of cold-water vibriosis in Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). *Appl. Environ. Microbiol.*, 1990, **56**, 1033–1037.
 34. Pedersen, K., Tiainen, T. and Larsen, J. L., Plasmid profiles, restriction fragment length polymorphisms and O-serotypes among *Vibrio anguillarum* isolates. *Epidemiol. Infect.*, 1996, **117**, 471–478.

Received 1 June 2015; revised accepted 28 October 2016

doi: 10.18520/cs/v112/i08/1749-1755

Opportunistic predatory behaviour in *Duttaphrynus melanostictus* (Schneider, 1799) tadpoles

Susmita Mahapatra¹, Sushil K. Dutta² and Gunanidhi Sahoo^{1,3,*}

¹Department of Zoology, North Orissa University, Baripada 757 003, India

²Plot No. 1573/01, Udyapuri (Gandamunda), Khandagiri, Bhubaneswar 751 030, India

³Present address: Department of Zoology, Utkal University, Bhubaneswar 751 004, India

We report *in situ* and *ex situ* observations on inter- and intra-specific predatory behaviour in tadpoles of the common Asian toad *Duttaphrynus melanostictus*. *In situ* *D. melanostictus* tadpoles feed on conspecific eggs, tadpoles of various developmental stages and adult carrion as well as dead heterospecific (*Fejervarya orissaensis* and *Euphlyctis cyanophlyctis*) tadpoles. Predation of weak, feebly swimming larvae and metamorphs in seminatural habitats under optimum conditions seems to be an opportunistic behaviour and diet enrichment, which needs additional support. Our observations support earlier reports indicating gradual desiccation, food shortage, competition and density as the probable factors of predation in temporary habitats.

Keywords: *Duttaphrynus melanostictus*, predation, tadpole, scavenger

PREDATORY behaviour is a widespread phenomenon in the animal kingdom. It is well documented among several anuran tadpoles (larvae) which demonstrate predatory interactions, including oophagy, cannibalism and necrophagy¹. It has been recorded in those species that breed in temporary ponds, ephemeral pools or puddles where they occur in high densities and are deprived of food². Cannibalism in natural or experimental conditions is quite common among tadpoles^{3,4}. Tadpoles of some species feed on conspecific eggs or tadpoles^{5–7}, while others prey upon heterospecific tadpoles^{8,9}. Factors such as food and space availability, microenvironment and mineral nutrients essential for metamorphosis shape the status of cannibalism in anurans¹⁰. Most cases of cannibalism involve oophagy^{3,4,11,12}, but occurrence of tadpole–tadpole cannibalism typically involves predation on different life stages¹.

The common Asian toad *Duttaphrynus melanostictus* is widely distributed in South and Southeast Asia; it breeds in both lentic (temporary and permanent pools) and lotic habitats (slow-flowing streams and canals). These tadpoles are gregarious and depending upon the circumstances, they may live as members of kin and/or mixed groups until metamorphosis¹³. We report predatory

*For correspondence. (e-mail: gunanidhi.nou@gmail.com)

behaviour of tadpoles of this species under both *in situ* and *ex situ* conditions, which has not been studied to the best of our knowledge. Our hypothesis is that *D. melanostictus* tadpoles predate only during unfavourable conditions.

The observations for the present study were made in the field during monsoon (August and September 2014) in northern Odisha, India. Permanent and temporary water bodies were surveyed four times weekly (8 a.m. to 3 p.m.) for the presence of tadpoles, after which the study sites were selected based on the presence of tadpoles (Table 1). Observations were made on the behaviour of the tadpoles till metamorphosis in these sites. Behavioural aspects on predation through visual scanning method were also drawn from resource-partitioning studies in half-filled semi-natural glass aquaria (2.5' × 1.5' × 1.0') ($n = 5$), each with 30 tadpoles (15 each of *D. melanostictus* and *Fejervarya orissaensis*). Food sufficiency in the aquaria was maintained through weekly addition of substrates collected from ephemerals. The Gosner stages¹⁴ reported for both predator and prey were based on naturalistic observations.

The metamorphosis period for the species was found to be 18–20 days in nature, which varied from 22 to 25 days under *ex situ* experimental conditions. A population of *D. melanostictus* tadpoles in an ephemeral pool (75' × 57' × 4' to 0.5') (average depth 12") at Sankhabhanga got fragmented into 17 small areas of about 1.5' × 0.7' × 0.5' within 7 days due to desiccation (no rainfall during that period) (Figure 1). Almost all the tadpoles were concentrated to these patches, which increased the density several fold due to desiccation. A total of 11 cannibalistic aggregations were observed in the patches that could be grouped into five categories (Table 1, Figure 2 a).

Two instances of oophagy in permanent ponds (Figure 2 b) and one instance of scavenging in a temporary pond (Figure 2 c) by several *D. melanostictus* tadpoles were also recorded during the study (Table 1). Heterospecific predation was also observed in an ephemeral pool involving *Euphlyctis cyanophlyctis* and *D. melanostictus* tadpoles. An *E. cyanophlyctis* tadpole (stage 35) eating a conspecific dead tadpole was attacked by 3–4 *D. melanostictus* tadpoles and driven away.

Ex situ heterospecific predation was recorded in one, out of five glass aquaria (2.5' × 1.5' × 1.0') with semi-natural laboratory experimental set-up. Five *D. melanostictus* tadpoles pursued and attacked a feebly swimming, weak *F. orissaensis* tadpole (two preyed on the tail, one on the gular region and two on the hind limbs). The prey surrendered after one minute (Figure 3). The metamorphs were seen hiding most of the time anticipating attack from the tadpoles.

Anuran larvae are known to exhibit plasticity in metamorphic traits in response to factors such as crowding, desiccation and predation pressure^{15–17}. Our observation on the metamorphic period of 22–25 days for *D. melanostictus*

is much lower than that of 31 days reported by Mogali *et al.*¹⁸. Both the observations were made under predator-free conditions, but Mogali *et al.*¹⁸ fed the tadpoles with boiled spinach, whereas our diet included food from natural substrates. Natural diet influences thyroid hormone function, which is essential for metamorphosis to occur¹⁹.

Interspecific predation among anurans, particularly predation by tadpoles on the eggs and hatchlings of other species may prove to be common. The predators in this situation gain nutrition while reducing or eliminating potential competition or predation. Carnivory, especially cannibalism, is common among anuran tadpoles that breed and develop in arid regions. Polis and Myers²⁰ listed 36 anurans known to exhibit intraspecific predation, where 11 species were reported to show tadpole–tadpole cannibalism and three oophagy. Hota and Dash²¹ performed an experiment on the competition between *Bufo melanostictus* and *Hoplobatrachus tigerinus* larvae, but it failed because the *H. tigerinus* larvae consumed many of the *Bufo* tadpoles in most of their treatments. Reports on larval cannibalism are available for *Scaphiopus bombifrons*²², *Bufo bores*²³, *Bufo marinus*²⁴, *Leptodactylus cunicularius*²⁵, *Hoplobatrachus tigerinus*²⁶ and *Duttaphrynus stomaticus*¹⁰. The temporary aquatic bodies undergo gradual desiccation towards the end of monsoon leading to increased larval density and food scarcity. Reduced food availability promotes predatory interactions among them that may act as a means of survival. The *D. melanostictus* tadpoles initiated cannibalistic behaviour and turned aggressive during such unfavourable periods. Large-sized tadpoles mostly preyed on weak conspecifics, metamorphs or juvenile ones, and then smaller members joined the attack. The prey–predator size relationship is variable irrespective of conspecific or heterospecific cannibalism. Predators may be smaller, equal or larger than the prey²³. The prey included eggs, metamorphs, similar-sized weak or dead tadpoles, or smaller but weaker individuals and even carrion. Heusser²⁷ speculated that conspecific oophagy may be a reason for synchronous oviposition and possibly synchronous



Figure 1. Patchy dense aggregation of *Duttaphrynus melanostictus* tadpoles in a desiccating temporary puddle.

Table 1. Records of predatory behaviour among anuran tadpoles in northern Odisha, India

Date of observation	Location	Time (h) of observation	Performers	Receiver(s)	Duration of event (min)	Observations
31 August 2014	21°54'09"N lat. 086°48'22"E long. (Sankhabhanga)	13:30	10-12 (stage 35-38)	Conspecific	120	Dead metamorph.
		13:55	9 (stage 36-38)	Conspecific	90	Similar-sized weakly swimming prey. Tail followed by hind limb were attacked.
		13:56	12-15 (stage 32-38)	Healthy conspecific metamorph	161	Attack initiated by one and followed by the arrival of others. Newly erupted limbs and gular regions consumed first.
8 September 2014 (13 aggregation observed)	21°58'36"N lat. 08°46'97"E long. (Rajabasa)	13:56	Three aggregations of 5-6	Four dead conspecifics	58	Larger tadpoles fed first.
		14:15	6	Single live conspecific (stage 26)	55	Two tadpoles observed holding the prey through the tail region. The attack lasted 2-3 min; five others joined after 15 sec.
		14:15		Conspecifics		
12 August 2014	21°58'36"N lat. 86°46'97"E long. (Ranibandh)	14:15	7-15	Single dead conspecific	35	Five aggregations preying on dead.
		14:40	12-15	Conspecific metamorph	52	Six groups feeding on metamorphs.
		15:00	10-12	Single live conspecific	126	Two groups eating on weak, feebly swimming small tadpoles.
21 September 2014	21°56'75"N lat. 86°47'51"E long. (<i>ex situ</i>)	15:35	10-12	Single dead Ec tadpole	145	Two Ec tadpoles feeding on a dead conspecific were over-powered by 3-4 Dm tadpoles. After 10 min an Ec tadpole tried to feed, but within a minute 5-6 Dm tadpoles started biting its tail and hind limbs at frequent intervals. This continued for 1-2 min that forced the Ec tadpoles to leave.
		14:20	5	Single live Fo tadpole	-	Two preyed on tail, one on gular region, and two on hind limbs.
		13:07	Hundreds	Conspecific egg	-	Almost all the tadpoles of a pond aggregated around conspecific eggs and began feeding.
31 July 2014	21°58'36"N lat. 86°46'97"E long. (Chenadua)	14:14	Many	Conspecific carcass	-	Adult carcass was eaten by a large number of tadpoles.

Dm, *Duttaphrynus melanostictus*; Ec, *Euphlyctis cyanophlyctis*, and Fo, *Fejervarya orissaensis*.

The predation sequences may be arranged date wise starting 11 July, 31 July, 12 August, 8 September and 21 September.

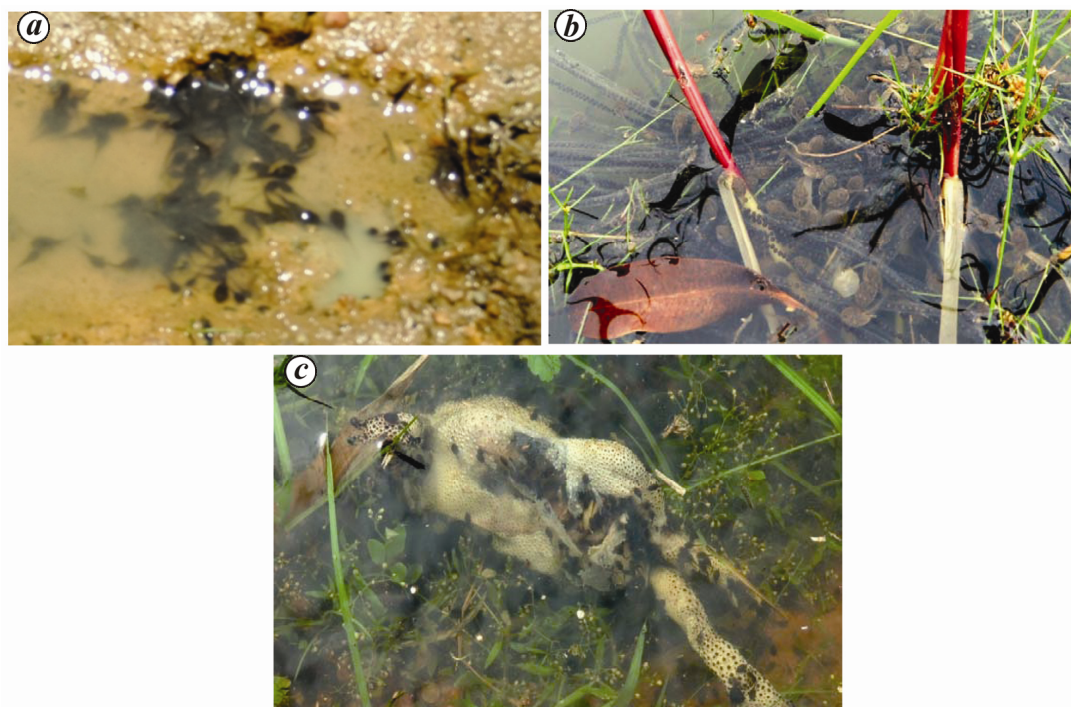


Figure 2. *D. melanostictus* tadpoles feeding on (a) a conspecific in the wild; (b) conspecific eggs in the wild; (c) adult conspecific carrion.

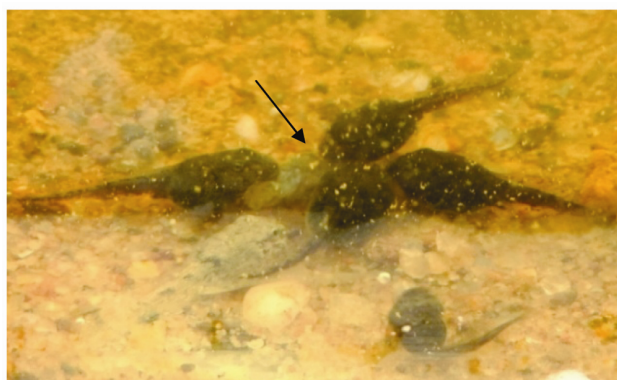


Figure 3. Four *D. melanostictus* tadpoles and one *Fejervarya orissaensis* tadpole eating a dead tadpole of the latter. Arrow indicates the prey.

metamorphosis. Reports of tadpole eating conspecific tadpole in the field have not always made it clear whether the prey were already dead before they were eaten. However, our observations include such information, both in the field and the laboratory, where live but weak tadpoles and metamorphs were eaten by tadpoles. We agree with Crump's²⁸ assumption that metamorphosing tadpoles and metamorphs are more vulnerable to predation because they have diminished locomotory capacity compared to tadpoles without forelegs or froglets without tails. In a heterospecific *D. melanostictus* (predator)–*E. cyanophlyctis* (prey) predation, the former was seen feeding on the latter that was at least seven times larger in size. The

gregarious nature of *D. melanostictus* and solitary nature of *E. cyanophlyctis* could have made this possible. Besides, in most cases, the tail is targeted first, followed by the gular region and newly emerged soft parts. Similar observations were made for *Bufo bores*²³, where the gular region or newly emerged limbs of conspecifics were attacked first. However, the predatory behaviour of *D. melanostictus* tadpoles on *F. orissaensis* individuals in glass aquaria was contrary to these observations (no desiccation, abundant natural food, ambient temperature and sunlight). This might be a typical opportunistic behavioural aspect of *D. melanostictus* tadpoles, which they continue even under favourable conditions. These observations add to the fact that larval cannibalism represents better alternatives to starvation and provides the best chance for growth with protein-rich diet, quicker development in a drying habitat and survival of the tadpoles. Additionally, cannibalism diminishes the level of competition among tadpoles by reducing population density^{29,30}. Estimates of the rate of occurrence of opportunistic cannibalism and cannibal morphotype are available for a few species, but in general little is known about the importance of cannibalism and carnivory in the diets of generalist tadpoles⁴. In a review of the evolution of cannibalism and cannibal morphotypes in amphibians, Polis and Myers²⁰ concluded that there was little evidence for genetic polymorphisms and much evidence for switches to cannibalism in response to environmental cues. Temporary aquatic habitats where tadpoles develop can quickly evaporate and tadpole density rapidly increases.

It may be necessary for individuals to adopt more aggressive competitive behaviour which could lead to cannibalism³¹.

The current observation supports the opportunistic predatory behaviour of *D. melanostictus* tadpoles. They feed on a wide range of food items, including plankton, leaves, eggs, detritus, carrion and tadpoles (cannibalism). This feeding ecology in tadpoles enabled the species to metamorphose quickly, survive and spread widely under most conditions throughout its distributional range. We assume that predation by *D. melanostictus* tadpoles even in abundant food, space and optimal environmental conditions is opportunistic, in response to the necessity of a protein-rich diet for faster metamorphic success; this particular aspect needs further studies.

1. Hoff, K. S., Blaustein, A. R., McDiarmid, R. W. and Altig, R., Behavior: interactions and consequences. In *Tadpoles: The Biology of Anuran Larvae* (eds McDiarmid, R. W. and Altig, R.), University of Chicago Press, Chicago, USA, 1999, pp. 215–239.
2. Crump, M. L., Opportunistic cannibalism by amphibian larvae in temporary aquatic environments. *Am. Nat.*, 1983, **121**(2), 281–289.
3. Crump, M. L., Cannibalism in amphibians. In *Cannibalism: Ecology and Evolution among Diverse Taxa* (eds Elgar, M. A. and Crespi, B. J.), Oxford University Press, Oxford, UK, 1992, pp. 256–276.
4. Alford, R. A., Ecology: resource use, competition and predation. In *Tadpoles: The Biology of Anuran Larvae* (eds McDiarmid, R. W. and Altig, R.), University of Chicago Press, Chicago, USA, 1999, pp. 240–278.
5. Muedeking, M. H. and Heyer, W. R., Descriptions of eggs and reproductive patterns of *Leptodactylus pentadactylus* (Amphibia: Leptodactylidae). *Herpetologica*, 1976, **32**, 137–139.
6. Hero, J. M. and Galatti, U., Characteristics distinguishing *Leptodactylus pentadactylus* and *Leptodactylus knudseni* in the Central Amazon rainforest. *J. Herpetol.*, 1990, **24**, 227–228.
7. Silva, W. R., Giaretta, A. A. and Facure, K. G., On the natural history of the South American pepper frog, *Leptodactylus labyrinthicus* (Spix, 1824) (Anura: Leptodactylidae). *J. Nat. Hist.*, 2005, **39**, 555–566.
8. Heyer, W. R., McDiarmid, R. W. and Weigmann, D. L., Tadpoles, predation and pond habitat in the tropics. *Biotropica*, 1975, **7**, 100–111.
9. Cardoso, A. J. and Sazima, I., Batracofagia na fase adulta e larvária darã-pimenta *Leptodactylus labyrinthicus* – Anura, Leptodactylidae. *Ciênc. Cult. (Sao Paulo)*, 1977, **29**, 1130–1132.
10. Neelam, S., Balwanand, W. K. and Tripathi, N. K., Larval cannibalism in the Indus valley toad, *Duttaphrynus stomaticus*. *Bull. Environ. Pharmacol. Life Sci.*, 2013, **2**(6), 148–150.
11. Duellman, W. E. and Trueb, L., *Biology of Amphibians*, McGraw-Hill, New York, USA, 1994, p. 670.
12. Escoriza, D., Predation of *Hyla intermedia* egg-clutches by tadpoles of *Discoglossus pictus* in Sicily. *Herpetol. Notes*, 2014, **7**, 575–576.
13. Saidapur, S. K. and Girish, S., The ontogeny of kin recognition in tadpoles of the toad *Bufo melanostictus* (Anura; Bufonidae). *J. Biosci.*, 2000, **25**, 267–273.
14. Gosner, K. L., A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 1960, **16**, 183–190.
15. Wilbur, H. M. and Collins, J. P., Ecological aspects of amphibian metamorphosis. *Science*, 1973, **182**, 1305–1314.
16. Travis, J., Anuran size at metamorphosis: experimental test of a model based on intraspecific competition. *Ecology*, 1984, **65**, 1155–1160.
17. Harris, R. N., The anuran tadpole: evolution and maintenance. In *Tadpoles: The Biology of Anuran Larvae* (eds McDiarmid, R. W. and Altig, R.), University of Chicago Press, Chicago, USA, 1999, pp. 279–294.
18. Mogali, S. M., Saidapur, S. K. and Shanbhag, B. A., Levels of predation modulate antipredator defence behavior and metamorphic traits in the toad *Bufo melanostictus*. *J. Herpetol.*, 2011, **45**(4), 428–431.
19. Kupferberg, S. J., The role of larval diet on anuran metamorphosis. *Am. Zool.*, 1997, **37**, 146–159.
20. Polis, G. A. and Myers, C. A., A survey of intraspecific predation among reptiles and amphibians. *J. Herpetol.*, 1985, **19**, 99–107.
21. Hota, A. K. and Dash, M. C., Evidence of interspecific predation among larval anurans: Predation of *Rana tigrina* larvae on *Bufo melanostictus* larvae. *Biol. Bull. India*, 1983, **5**(1), 54–55.
22. Pfennig, D. W., Reeve, H. K. and Sherman, P. W., Kin recognition and cannibalism in spadefoot toad tadpoles. *Anim. Behav.*, 1993, **46**, 87–94.
23. Jordan, D. J., Rombough, C. J., Pearl, C. A. and McCreary, B., Cannibalism and predation by western toad (*Bufo boreas boreas*) larvae in Oregon, USA. *Western N. Am. Nat.*, 2004, **64**(3), 403–405.
24. Pizzatto, L. and Shine, R., The behavioral ecology of cannibalism in cane toads (*Bufo marinus*). *Behav. Ecol. Sociobiol.*, 2008, **63**, 123–133.
25. Pirani, R. M., da Silva, E. T. and Feio, R. N., Tadpole cannibalism in *Leptodactylus cunicularius* Sazima & Bokermann 1978 (Anura: Leptodactylidae) at a temporary stream in South-eastern Brazil. *Herpetol. Notes*, 2010, **3**, 359–360.
26. Rajput, P. A., Shanbhag, A. B. and Saidapur, S. K., Absence of kin discrimination in cannibalistic anuran tadpoles of *Hoplobatrachus tigerinus* (Daudin). *Int. J. Exp. Biol.*, 2011, **49**, 362–365.
27. Heusser, H., Spawn eating by tadpoles as possible cause of specific biotope preferences and short breeding times in European anurans (Amphibia Anura). *Oecologia*, 1970, **4**, 83–88.
28. Crump, M. L., Cannibalism by younger tadpoles: another hazard of metamorphosis. *Copeia*, 1986, **4**, 1007–1009.
29. Bleakney, S., Cannibalism in *Rana sylvatica* tadpoles, a well-known phenomenon. *Herpetologica*, 1958, **14**, 34.
30. Meffe, G. K. and Crump, M. L., Possible growth and reproductive benefits of cannibalism in the mosquito fish. *Am. Nat.*, 1987, **129**, 203–212.
31. Jefferson, D. M. and Demuth, B. S., The conditional benefits of cannibalism for wood frog tadpoles (*Lithobates sylvaticus*). *Can. Sci.*, 2014; doi:#10.13034/14-018.

ACKNOWLEDGEMENTS. S.M. and G.S. thank the Science and Engineering Research Board, Government of India for financial support (SR/S/AS-26/2012). We thank K. Vasudevan and G. Prudhviraj for their comments that helped improve the manuscript.

Received 18 August 2015; revised accepted 19 November 2016

doi: 10.18520/cs/v112/i08/1755-1759