Do male tree frogs feed during the breeding season? Stomach flushing of five syntopic hylid species in Rio Grande do Sul, Brazil

MIRCO SOLÉ & BIRGIT PELZ

Laboratory of Biological Research (LPB), Pontificia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Rio Grande do Sul, Brazil and Zoological Institute, University of Tübingen, Tübingen, Germany

(Accepted 29 August 2007)

Abstract
The diet of males of five syntopic hylid species from the Araucaria plateau of Rio Grande do Sul was studied by stomach flushing. Because the duration of their breeding seasons was rather different, the levels of food intake at the respective breeding sites were analysed. We found a positive correlation between the length of the male calling period and the ingestion of prey. The longer the species-specific reproductive activity of the males, the more individuals fed during that time. The results are discussed under congruence aspects of feeding and mating microhabitats in these tree frogs. In addition, differences in niche breadth may influence the temporal and spatial partitioning between the species studied and result in quantitative as well as qualitative patterns of prey consumption, thereby ensuring a maximum of fitness gain.

Keywords: Anura, Araucaria plateau, breeding season, calling and feeding activity, Hylidae, southern Brazil.

Introduction
Many tree frog species (Hylidae) have to leave their feeding habitats for mating because reproduction is linked to water (e.g. Donnelly and Guyer 1994; Haddad and Sawaya 2000; Haddad and Prado 2005). For that reason they may have to travel some distance to reach suitable water bodies (Richter et al. 2001; Regosin et al. 2005). The duration of the breeding season differs among species and depends on factors such as climate and reproductive behaviour (Melchiors et al. 2004; Toledo and Haddad 2005; Zina and Haddad 2005). In the subtropical northeast of Rio Grande do Sul, Brazil, the Araucaria plateau has to be regarded as a hotspot of anuran diversity (Kwet 2001), in particular hylids. Thirty-four of the 76 recorded species are tree frogs (Kwet 2004). Remarkable
differences in reproductive patterns have been found, particularly in syntopic species, ranging from prolonged mating periods to explosive breeders (Kwet 2001).

Mating activity entails high costs, especially for males. Calling not only requires much energy (Wells and Taigen 1986) but also increases the risk of predation (Ryan et al. 1981; Lahanas 1995). However, focusing on hunting to compensate for the energy loss may negatively affect mating success. Hence males of species with short reproductive periods may interrupt food intake for that time and concentrate on breeding efforts. Therefore, a correlation between feeding behaviour and duration of the calling season is assumed. In order to test this hypothesis, the present study included the following steps:

1. Selection of syntopic hylid species with different patterns of reproduction.
2. Capture of 50 calling males per species.
3. Collection of their stomach content by flushing.
4. Identification of the items found in the stomachs.
5. Compilation of species-specific male diets.
6. Testing a correlation between duration of the reproductive period and male feeding.

**Materials and methods**

Our study was carried out at the Centro de Pesquisas e Conservação da Natureza Pró-Mata, located at altitudes of about 1000 m on the Serra Geral of Rio Grande do Sul, Brazil (29°S, 50°W). The area of this reserve comprises 5000 ha of Araucaria rain forest, secondary vegetation, and some open grassland (Heinle 2002). The field studies took place in spring and early summer from November 2002 to January 2003, within the rainy season at this subtropical site.

Five abundant hylid species with different reproductive patterns were chosen: *Hypsiboas faber* (Wied-Neuwied, 1821) (main calling activity about 2 months per year) (Martins 1993; Kwet 2001), *Dendropsophus minutus* (Peters, 1872) (about 4 months per year) (Kwet 2001; Vasconcelos and Rossa-Feres 2005), *Hypsiboas pulchellus* (Duméril and Bibron, 1841) (about 8 months per year), and *Scinax granulatus* (Peters, 1971) and *Scinax perereca* Pombal, Haddad, and Kasahara 1995 (each about 3 months per year) (Kwet and Di-Bernardo 1999; Kwet 2001).

Fifty calling males of each species were captured manually and their stomach contents examined. The frogs were collected during their mating season, at night at their calling sites. Within 3 h their stomachs were flushed in the laboratory by a simple method that allows retrieving the stomach contents without harming the frogs (Solé et al. 2002, 2005). After that procedure animals were released at the sites of capture. Stomach contents were preserved in 70% ethanol and later identified at least to order level under a stereomicroscope using the keys of Triplehorn and Johnson (2005). Pearson’s $\chi^2$ test was applied for statistical analysis.

**Results**

The 250 male frogs treated by stomach flushing all survived. During their breeding seasons, we found empty stomachs as well as those containing various items, with striking differences between the five species (Figure 1). As an overall result, *Hypsiboas pulchellus* had
about twice more filled stomachs (84%) than those of the other four species (38–48%). The \( \chi^2 \) test shows a significant difference (\( P<0.01 \)).

The stomach content was composed of animal prey and/or plant remains. Animal items were most frequently found in stomachs of Hypsiboas pulchellus, but only rarely in Hypsiboas faber (Figure 2). Again this difference was significant (\( \chi^2 \) test, \( P<0.001 \)). The few animal prey ingested by adult males of the latter species were Opiliones, larval Lepidoptera, and other anurans. Dendropsophus minutus mostly fed on Araneae, Diptera, and Homoptera. Hypsiboas pulchellus, the only species with a regular intake of animal prey, mostly fed on Coleoptera, larval Lepidoptera, and Hymenoptera. Coleoptera and Homoptera were the animal taxa mainly ingested by Scinax granulatus, whereas S. perereca fed on Araneae and Blattaria (Table I).

Quite often plant material was found in the frogs’ stomachs; it was observed for all species (Figure 3) without any significant difference (\( \chi^2 \) test, \( P=0.7 \)).
The length of the calling period varied in the hylids studied between two and eight months per year. Correspondingly, the number of male frogs with ingested animal prey increased from four to 42 (Figure 3).

Discussion

The sample of 50 calling males per species can be considered to reflect their average feeding activity during the mating period (Solé et al. 2005).
At the study site, Pró-Mata, tree frogs inhabit quite different types of vegetation, ranging from Araucaria and angiosperm tree stands, various stages of succession of the surrounding secondary vegetation to open grasslands (Engels et al. 2002). Many temporary and permanent ponds are found in the grassland, and are used for spawning (Kwet 2001). The males of most of the species studied have to cross some distance between their feeding and calling habitats. In particular, those living in dense forests have to migrate across open grassland in order to reach suitable water bodies where they can attract mature females. Of course this increases the costs of reproduction. According to the hypothesis, during resulting short breeding periods the males should concentrate on calling and not on feeding activities. In fact the data on stomach content of the 250 hylid males, flushed during their breeding season, clearly support this hypothesis (Figure 1). In all five species studied a significant correlation was found between the duration of the breeding season and the intake of animal prey (Figure 3): the longer the reproductive period, the more male frogs feed during that time. This may also increase the fitness by minimizing the costs of reproduction and optimizing the use of local resources.

The present samples provide a better understanding of the diet of calling males. For example, out of 50 examined Hypsiboas faber males, the species with the shortest breeding season, only four had animal prey items in their stomach. Probably, in this species the males do not search for prey at all in their breeding territory, but concentrate on advertisement calls because of local competition with conspecifics (Kime et al. 2000). Perhaps the four individuals with some food in the stomach had only recently arrived at the pond where they were captured, or the prey items were ingested by chance. On the other hand, nearly all males of Hypsiboas pulchellus had animal items in the stomach while resident at the breeding site. This species, however, inhabits open grassland areas and not forests (Di-Bernardo et al. 2004), suggesting a large congruence of feeding and mating habitats. The males may alternate both activities, as has previously been recorded for Engystomops pustulosus (Cope, 1864) in Panama (Ryan 1985) and for three sympatric species of anurans in the USA (Anderson et al. 1999). In a population of Hypsiboas pulchellus from Uruguay, 33% of the examined frogs had empty stomachs (Maneyro and da Rosa 2004). This percentage is greater than in the present research, but is still far less than the percentages found for the other three species examined in the present study. The percentage of empty stomachs found for H. pulchellus during this study is comparable to that found during other dietary studies: Van Sluys et al. (2001) studied the diet of the leptodactylid frog Zachaenus parvulus (Girard, 1853), a leaf-litter inhabitant, and found 8.6% empty stomachs. Maneyro et al. (2004) found 21% of all specimens with empty stomachs in Leptodactylus ocellatus (Linnaeus, 1758) during a pitfall survey in Uruguay. The three other hylids studied here are all forest inhabitants (Kwet 2001), so the males had to migrate some distance to the ponds in the open land nearby.

Plant material (mostly leaves) was identified in the stomach of males of all five hylids studied. Assuming that pieces of plant are occasionally ingested along with animal prey (Korschgen and Moyle 1955; Brandão et al. 2003), but remain in the stomach for quite a long time due to lack of digestion, these remains might not indicate recent feeding.

**Acknowledgements**

We would like to thank Simone Schromm and Olaf Beckmann for assistance in the field and in the laboratory. The PUCRS and especially the staff of the Instituto de Meio
Ambiente provided valuable support for our studies in the Pró-Mata reserve. We appreciate the critical reading of the manuscript by Wolf Engels, Axel Kwet, and Michael Kroniger. Stefan Lötters and an anonymous referee made helpful comments. This study is part of the projects DLR-BMBF 01LT0011/7 “Araucaria forest” and DLR/IB-FAPERGS “Subsidios para o diagnóstico ambiental do Planalto das Araucárias”. This article is dedicated to the memory of the late Marcos Di-Bernardo.

References

Anderson AM, Haukos DA, Anderson JT. 1999. Diet composition of three anurans from the Playa Wetlands of Northwest Texas. Copeia 1999:515–520.
Brandão RA, Garda A, Braz V, Fonseca B. 2003. Observations on the ecology of Pseudis bolbodactyla (Anura, Pseudidae) in central Brazil. Phyllomedusa 2(1):3–8.
Di-Bernardo M, Oliveira RB, Pontes GMF, Melchiors J, Solé M, Kwet A. 2004. Anfibios anuros da região de extração e processamentos de carvão de Candidia, RS, Brasil. In: Teixeira EC, editor. Estudos Ambientais em Candidia: Carvão e seus impactos. (Brazil): FINEP/PDACT/CIAMB/FAPERGS/FEPM. p 163–175.
Donnelly MA, Guyer C. 1994. Patterns of reproduction and habitat use in an assemblage of neotropical hylid frogs. Oecologia 98:291–302.
Engels W, Hamp R, Tschupke W. 2002. BMBF Project Araucaria forest. Reforestation of a highly endangered subtropical rainforest ecosystem, originally covering large areas of the Southern Mata Atlântica. Pró-Araucária online. http://www.uni-tuebingen.de/entw-phys/CD/Evaluation.pdf.
Haddad CFB, Prado CPA. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. BioScience 55:207–217.
Haddad CFB, Sawaya RJ. 2000. Reproductive modes of Atlantic forest hylid frogs: a general overview and the description of a new mode. Biotropica 32:862–871.
Heinle S. 2002. Schlussbericht über die 1. Phase des Forschungsproektibes Araukarienwald. Pró-Araucária online. http://www.stz-rottenburg.de/easylit/modul15/html/schlussbericht.html.
Kime NM, Turner WR, Ryan MJ. 2000. The transmission of advertisement calls in Central American frogs. Behavioral Biology 11:71–83.
Korschgen LJ, Moyle DL. 1955. Food habits of the bullfrog in central Missouri farm ponds. American Midland Naturalist 54:332–341.
Kwet A. 1999. Froße im brasilianischen Araukarienwald: Anurengemeinschaft des Araukarienwaldes von Rio Grande do Sul: Diversität, Reproduktion und Ressourcenaufteilung. Münster: Natur und Tier-Verlag.
Kwet A. 2004. Froße im brasilianischen Araukarienwald. Biologie in Unserer Zeit 34:170–178.
Kwet A, Di-Bernardo M. 1999. Pró-Mata—Anfibios, Amphibien, Amphibians. Porto Alegre (Brazil): EDIPUCRS.
Lahanas PN. 1995. The function of near neighbors in decreasing call latency period by the Tungara frog, Physalaemus pustulosus. Biotropica 27:262–265.
Maneyro R, da Rosa I. 2004. Temporal and spatial changes in the diet of Hyla pulchella (Anura, Hylidae) in southern Uruguay. Phyllomedusa 3:101–113.
Maneyro R, Naya DE, da Rosa I, Canavero A, Camargo A. 2004. Diet of the South American frog Leptodactylus ocellatus (Anura, Leptodactylidae) in Uruguay. Iheringia, Série Zoologia 94:57–61.
Martins M. 1993. Observations on the reproductive behaviour in the Smith Frog, Hyla faber. Herpetological Journal 3:31–34.
Melchiors J, Di-Bernardo M, Pontes GMF, Oliveira RB, Solé M, Kwet A. 2004. Reprodução de Pseudis minuta (Anura, Hylidae) no sul do Brasil. Phyllomedusa 3:61–68.
Regosin JV, Windmiller BS, Homan RN, Reed M. 2005. Variation in terrestrial use by four pool-breeding amphibian species. Journal of Wildlife Management 69:1481–1493.
Richter SC, Young JE, Seigel RA, Johnson GN. 2001. Postbreeding movements of the Dark Gopher Frog, Rana setos a Goin and Netting: implications for conservation and management. Journal of Herpetology 35:316–321.
Ryan MJ. 1985. The Túngara frog: a study in sexual selection and communication. Chicago: University of Chicago Press.
Ryan MJ, Tuttle MD, Taft LK. 1981. The costs and benefits of frog chorusing behavior. Behavioral Ecology and Sociobiology 8:273–278.
Solé M, Beckmann O, Pelz B, Kwet A, Engels W. 2005. Stomach-flushing for diet analysis in anurans: an improved protocol evaluated in a case study in Araucaria forests, southern Brazil. Studies on Neotropical Fauna and Environment 40:23–28.

Solé M, Ketterl J, Di-Bernardo M, Kwet A. 2002. Ants and termites are the diet of the microhylid frog Elachistocleis ovalis (Schneider, 1799) at an Araucaria forest in Rio Grande do Sul, Brazil. Herpetological Bulletin 79:14–17.

Toledo LF, Haddad CFB. 2005. Reproductive biology of Scinax fuscomarginatus (Anura: Hylidae) in south-eastern Brazil. Journal of Natural History 39:3029–3037.

Triplehorn CA, Johnson NF. 2005. Borror and Delong’s introduction to the study of insects. 7th ed. Belmont (CA): Thomson Brooks/Cole.

Van Sluys M, Rocha CFD, Souza MB. 2001. Diet, reproduction, and density of the leptodactylid litter frog Zachaeus parvulus in an Atlantic rain forest of southeastern Brazil. Journal of Herpetology 35:322–325.

Vasconcelos TS, Rossa-Feres DC. 2005. Diversidade, distribuição espacial e temporal de anfíbios anuros (Amphibia, Anura) na região noroeste do estado de São Paulo, Brasil. Biota Neotropica 5:137–150.

Wells KD, Taigen TL. 1986. The effect of social interactions on calling energetics in the gray treefrog (Hyla versicolor). Behavioral Ecology and Sociobiology 19:9–18.

Zina J, Haddad CFB. 2005. Reproductive activity and vocalization of Leptodactylus labyrinthicus (Anura: Leptodactylidae) in southeastern Brazil. Biota Neotropica 5:119–129.