

A curious case of herbivory in the common toad *Rhinella arenarum arenarum* during hibernation in captivity conditions

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The common toad *Rhinella arenarum arenarum* is used in our laboratory for teaching and research, studying different aspects of the anuran development. Hormonally induced adults allow us to obtain embryos by *in vitro* fertilization throughout the year. However, adult toads hibernate during nonbreeding season (March-August) making it very hard to find them in nature (Gallardo, 1958). Consequently, we usually maintain captive breeding pairs after breeding season, inducing an “artificial winter” in the laboratory. The finding described in this note was observed on August 2012 in a group of animals that came out of their artificial hibernation.

Toads of *R. arenarum arenarum* (three males and six females) were collected during the first week of March 2012 from semi-natural sites in the surroundings of the campus of the Universidad de Buenos Aires (34° 32' S- 58° 26' O). We maintained the animals in a communal clean plastic container (55 x 45 x 40 cm) under controlled laboratory conditions (photoperiod= 14:10h light:dark and temperature= 26-28°C). A small pool filled with water (18 x 15 x 7 cm), to which toads could freely access, was placed in the center of the container. Animals were fed daily with live crickets. After a period of about 40 days under the conditions described above, the animals that were not used for research were artificially induced to hibernate: An adult male (Snout-vent length, SVL= 104 mm), two adult females (SVL= 116 and 125 mm) and a young female (SVL= 88 mm) were transported to a semi-darkness area in the laboratory and maintained at 18-20°C during seven days. Toads were not fed or disturbed during this period. Then, the animals were put in a polypropylene gunny sack with wet grass and transferred into a cardboard box in a refrigerated room (8-10°C). On the morning of August 13, 2012 (after about three months), the animals were taken from their hibernation and transferred to artificial spring conditions (14:10 h light:dark; 28-30°C) within the container previously

used. Moreover, we added a steady trickle of water over the small central pool simulating a rainy day. After some hours in these conditions, animals became notoriously active. Before the end of the first day we approached the animals to offer them some crickets, but three pieces of excrement attracted our attention. Curiously, the three feces (of about 3-4 cm length) were abundantly composed of vegetable matter unified by a mud-like substance. We fixed them in ethanol 70° and photographed them with a digital camera (Fig. 1A). Two of the three feces were disaggregated and examined under a stereo microscope. Surprisingly, we confirmed that they were entirely composed by undigested or partially digested pieces

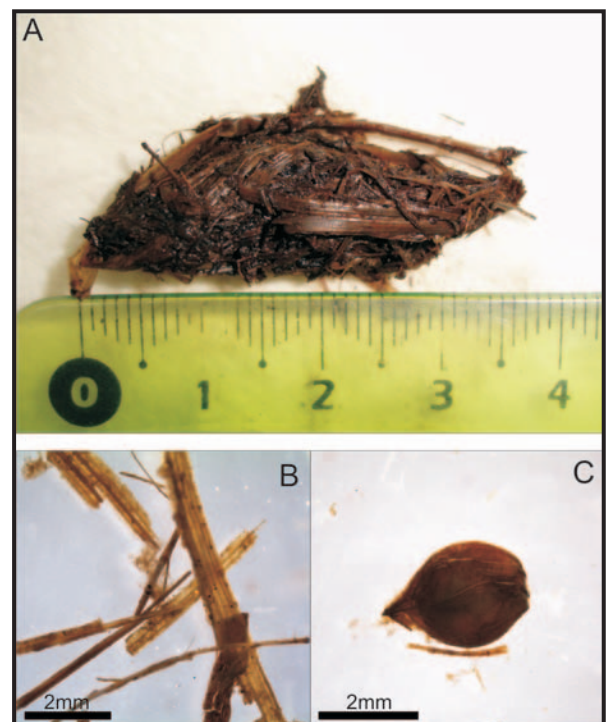


Figure 1. A) Feces of *R. arenarum arenarum* containing a large quantity of vegetable material, the mud-like substance that mixed it was considerably loose during fixation processes. Stereo microscope analysis demonstrates that feces were entirely composed by partially digested graminoids (B), and even some seeds were found (C).

of graminoids (Fig. 1B), and even some seeds were found (Fig. 1C). As the animals diet was controlled for almost two months before artificial hibernation induction, the most likely explanation is that animals had eaten the grass that was used as substrate during hibernation. However, this would imply a striking behavior since dormant amphibians usually cease all feeding and other activities during this period (Wells, 2007). Unfortunately, since the animals were kept in groups all the time, we do not know if the feces came from one or more individuals.

The vast majority of anurans feed primarily on insects and other arthropods (Duellman and Trueb, 1986). However, the discovery of plant materials in the diet content of anurans has been cited in numerous studies. In most anurans species, the presence of vegetable matter in the diet content is generally assumed to be ingested accidentally (Gallardo, 1958, 1975; Basso, 1990; Lajmanovich, 1994; Hirai and Matsui, 1999). In *R. arenarum arenarum* and *R. schneideri* the presence of plant debris and seeds in the diet content has been interpreted as a consequence of leafcutter ants consumption (*Acromyrmex* spp.), which carry traces of vegetation (Gallardo, 1975; Lajmanovich, 1994). However, although registers of herbivory are scant in the literature, adult anurans of some species ingest sufficient vegetation to be considered omnivorous or herbivorous. For example: The hylid frog *Xenohyla truncata* includes the fruit of several species of plants in its diet (Da Silva and De Britto-Pereira, 2006), the green pond frog *Euphlyctis hexadactylus* feeds on aquatic vegetation (Das and Coe, 1994), and the toad *Rhinella marina* may eat vegetable scraps and canned dog food (Alexander 1964).

Several factors indicate that our findings may not be a case of an accidental vegetable ingestion: 1) There were not insects or another macroscopic arthropods inside the gunny sack (which was tightly knotted) that toads could hunt; 2) The refrigerated room was completely dark, so that no hunting behavior could be triggered by any insect-like moving

object; and 3) Except for the mud-like substance, feces was entirely composed of plant material. Interestingly, field data indicate that males and females of *R. arenarum arenarum* hibernate in shallow horizontal caves with leaves inside (Gallardo, 1958). It would be interesting to perform field experiments to elucidate whether vegetable consumption occurs in nature during the hibernating period of the animals; or, whether the findings described here were an unusual behavior due to captive conditions.

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