Functional necrophilia: a profitable anuran reproductive strategy?

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Explosive breeding is a common reproductive strategy, especially in anurans. In some cases of explosive breeding in anurans, intense aggressive interactions occur among males attempting to gain access to females, and for terrestrial species that mate in water, females may die by drowning during long mating struggles. Such occurrences are obviously detrimental to females, and may also cause a decrease in the reproductive success of males through depletion of energy without obtaining access to a live female. However, the males of the small Amazonian frog \textit{Rhinella proboscidea} can promote the ejection of oocytes from the abdominal cavities of dead females and fertilize them. This behaviour can minimize losses to both partners during explosive reproduction events. The existence of such a “functional necrophile strategy” shows that there may be possible selection in favour of stronger and more persistent males in explosive breeders.

\textbf{Keywords:} amphibians; davian behaviour; reproduction; mating balls

\textbf{Introduction}

A reproductive behaviour or strategy may be viewed as the combination of physiological, morphological and behavioural traits that act together to maximize the number of offspring produced under given environmental conditions (Stearns 1992). Different behaviours can increase the efficiency (fitness) or minimize the costs and risks associated with reproductive processes (Harvey and Pagel 1991; Stearns 1992; Sakai and Westneat 2001). Consequently, there are a myriad of different strategies among animals, including some that are considered “socially unacceptable” and impossible in human society, such as infanticide in insects and vertebrates (Hager and Johnstone 2004), necrophilia in lizards (Costa et al. 2010) and amphibians (this study) and post-mating clutch piracy (Vieites et al. 2004).

In general, the reproductive behaviour of animals can be divided into two basic temporal patterns: prolonged breeding and short-term “explosive” breeding (see Wells...
1977, 2007; Odendaal et al. 1985). Explosive reproduction is common in some animal groups, and is particularly well studied in some anuran families, such as Bufonidae, Hylidae, Microhylidae and Ranidae (Wells 1977, 1979; Zimmerman and Bogart 1988; Prado et al. 2005; Rodrigues et al. 2004, 2005, 2007). Explosive breeding is generally characterized by males remaining at breeding sites for only a few days, chorus formations, synchronous arrival of the female in the pond, and low selectivity of males (active search for female) (Wells 1977). The formation of ponds, amount of rainfall, larval competition and predator abundance at breeding sites, demographic factors such as age at first reproduction and frequency of reproduction of individual females could have led to the evolution of explosive breeding (Wells 1977; Aichinger 1987; Menge and Olson 1990; Prado et al. 2005). The males of species with explosive breeding generally form large mating aggregations. In this situation, the females are available for a short time (Menin et al. 2006) and the males occur in higher density. Hence, the opportunity for female choice should be limited by intense male–male competition (Wells 2007). Indeed, the males use a lot of energy searching for mates and struggling over females (Wells 1977, 2007). Unpaired males try to displace those already in amplexus, resulting in large mating balls and this behaviour is sometimes fatal to females (Verrell and McCabe 1986; Menin et al. 2006; Wells 2007). For some of these species, females may be drowned under the weight of several males attempting to mate (Duellman and Trueb 1994; Trauth et al. 2000; Wells 2007). Such occurrences imply a loss of fitness for males as well as females because if reproductive contests between males take too long, and females die, there is a decrease in the reproductive success of males through loss of energy in fights that do not result in reproduction. In this case, the evolution of a behaviour trait that allows this energy and fitness loss to be minimized should be favoured. *Rhinella proboscidea* (Anura: Bufonidae) is a small central Amazonian frog that exhibits intense male–male competition for mating opportunities. This species is an explosive breeder, and up to several hundred males congregate in small streamside ponds or headwaters of streams for 2 to 3 days (Lima et al. 2006; Menin et al. 2006; Rodrigues et al. 2010), where thousands of fertilized eggs are laid (Figure 1A). Here we show that males of *R. proboscidea* can extract and fertilize oocytes from dead females, which is a previously undescribed reproductive strategy for amphibians.

**Material and methods**

**Study area**

This study was conducted between June 2001 and June 2005 during the rainy seasons in the primary forest of Reserva Florestal Ducke (between 02°55' and 03°01' S, 59°53' and 59°59' W), adjacent to the city of Manaus, Amazonas state, Brazil. The reserve covers 10,000 ha of *terra-firme* rain forest, a well-drained forest not subject to seasonal inundation. The forest is characterized by a 30-m to 37-m tall closed canopy, with emergent trees growing to 40-45 m (Ribeiro et al. 1999). The understorey contains abundant sessile palms (*Astrocaryum* spp. and *Attalea* spp.; Ribeiro et al. 1999). The climate is characterized by a rainy season from November to May and a dry season from June to October (Marques-Filho et al. 1981). Mean annual temperature is approximately 26°C (Marques Filho et al. 1981) and mean annual rainfall was 2489 mm between 1985 and 2004 (unpublished data from the Meteorological station of Reserva Ducke).
Methods

Observations of reproductive events were made sporadically in temporary ponds in headwater streams distributed over an area of 64 km² (Lima et al. 2006). Fifteen dead females were collected (10 females in June 2001 and five females in June 2005) in two different ponds during explosive reproductive events in which dozens of individuals had gathered for reproduction. All dead individuals collected were fixed in formalin and preserved in 70% alcohol. The females were dissected to check for the presence of mature ovarian oocytes. Observations of behaviour of amplexant pairs were made simultaneously with collection of dead individuals. Oocytes extracted and fertilized by males from dead females were maintained in transparent plastic basins until embryos and the developmental stage were defined following Gosner (1960). As the eggs of R. proboscidea are relatively large (c. 1.5 mm) we could observe their development in the field without removing the eggs from plastic bags, using a (10 ×) magnifying glass. In all cases we observed at least half the eggs in a clutch. The eggs were observed until they reached stage 13 by which stage fertilization is certain (Gosner 1960); we then fixed the eggs in 10% formalin. The embryos were fixed in the field because the team did not have the facilities needed to allow continued development in the laboratory. The stage of development was confirmed later in the laboratory under a stereomicroscope. Voucher specimens were deposited in the Coleção Zoológica Paulo Bührnheim of the Universidade Federal do Amazonas (CZPB-UFAM 279, lot 141) in Manaus, Amazonas, Brazil.

Results and discussion

Twenty dead R. proboscidea females were found in the first aggregation, which involved about 100 males. Only 10 dead females were collected and dissected. The other 10 showed some decomposition and were not collected. During the second aggregation, involving about 50 males, we found five dead females. Dissection revealed that none of the 15 females contained abdominal oocytes. In the first aggregation (Figure 1B) we witnessed a male in axillary amplexus with one dead female (Figure 1C), and he extracted her oocytes by squeezing the sides of her belly with rhythmic movements of his front and hind limbs (Figure 1C). We did not check the fertilization of eggs because of lack of materials suitable for observations in the field. During the second reproductive event, four R. proboscidea males were seen extracting oocytes from dead females. Three of them were not interrupted by other males but, on one occasion, a male was observed pushing the amplexant dead female around the pond, apparently to avoid other males. This male continued to compress the dead female’s abdomen during such displacements and the oocytes kept being expelled. All eggs were collected and maintained until they reached embryo stage 13 of Gosner (1960), so confirming fertilization. Fertilization in R. proboscidea is external and clutches of this species are bead-like gelatinous strings, with eggs disposed uniserially (Menin et al. 2006). This feature may make it easier for males to expel oocytes from dead females when compared with other groups such as hylids because of the provision of the eggs in the abdominal cavity.

Some females were observed leaving the pond after the second reproductive aggregation, indicating that some females survived the reproductive event. However, it is unknown if these females can survive and reproduce in the next breeding season. Necrophilic behaviour by males can, at least, minimize the female’s loss of investment
Figure 1. Necrophilia in *Rhinella proboscidea* in a central Amazonian headwater stream. Thousands of eggs (arrow) from a single reproductive event, in a small patch of a headwater stream (A). Two males in a battle for a drowned female. The larger (arrowed) is in amplexus and compressing the female’s abdomen with his legs, which resulted in expulsion of the oocytes (B). Male compressing the abdomen of a dead female, which resulted in expulsion of her oocytes (C).
in oocytes if she dies during a mating aggregation. Once operational sex ratio is
strongly biased to males (about ten males to one female; M. Menin, unpublished
data), and the males spend a lot of energy in these battles (Beebee 1996), the proba-
bility of a given male finding and mating with another female, once the first female
is dead, is probably small. It is therefore advantageous for the male to take advantage
of oocytes from dead females. It brings to light the hypothesis that this low probabil-
ity imposes a pressure on males to successfully mate with the first female found.
Selection should favour behaviour in mating males that does not culminate in the
death of females, unless some strategy is possible that minimizes the loss to the mat-
ing male’s fitness when the female’s death occurs. We suggest that the “necrophilia
strategy” could solve the question of why there should be selection for stronger and
persistent males (Duellman and Trueb 1994) if this reduces the number of live females
available to a persistent male, and why there should be selection for morphology such
as male size, keratinized spines on thumb and chest or development of arms as in
some bufonid species, that facilitates dominance over the female’s body, whether she
is dead or not (Greene and Funk 2009), independent of selection for female traits.
In the case of necrophilia, where there can be no future investment by the female,
partial oviposition of a clutch is obviously of no selective advantage. As expulsion of
oocytes from dead females probably can have fitness advantages for both partners,
the behaviour may be more prevalent in anurans, or even other groups that rely on
external fertilization, than present records indicate. However, studies of reproductive
behaviour of species that are explosive breeders should be conducted to confirm this
hypothesis.

Necrophilia has been reported in several vertebrate groups (mammals, birds,
amphibians and reptiles; Costa et al. 2010). Although necrophilia has been reported in
other species of anurans (Meshaka-Jr 1996; Bettaso et al. 2008; Sinovas 2009), this may
be the first case where the necrophilia brings a direct fitness gain, generating descen-
dants. In contrast to the conclusions of other studies (Sinovas 2009; Costa et al. 2010),
necrophilia is not a behavioural mistake in R. proboscidea, but rather is a functional
behaviour in terms of fitness, with positive effects on the reproductive success of both
males and females.

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