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Reproductive behavior of *Acanthagrion truncatum* Selys, 1876 (Odonata: Coenagrionidae)

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Behavioral data on Neotropical coenagrionids is still scanty, with very few studies on their reproductive behavior. Here we present the first description of the reproductive behavior of *A. truncatum* in a high density population in the Brazilian Neotropical savanna. The observations were made at a pond in an ecological reserve. Males remain at the water searching for females. Females remain in the surrounding vegetation and only approach the water to mate and oviposit. The mean duration of copulation was 25.6 ± 3.26 minutes. Copulations are concentrated between 12:00 and 14:00 h (71%). Females oviposit in tandem with males, sometimes submerging to oviposit. Oviposition took 43.08 ± 22.17 minutes. Female underwater oviposition seems to disrupt male guarding and females emerge from the water by themselves. Male–male interactions usually consist of chases and “facing off”. This damselfly species is apparently non-territorial, since males did not defend resources and searched for females in the area.

**Keywords:** Odonata; dragonfly; mating; sexual behavior; damselfly; Neotropics; Brazil

### Introduction

Reproductive behavior in Coenagrionidae damselflies presents many types and variations. Mean copulation duration may range from eight minutes in *Erythromma lindenii* to 395 min in *Ischnura senegalensis* (Cordoba-Aguilar et al., 2009, and references therein). Usually, damselflies are polygamous (e.g. Miller, 1987). However, in some species individuals copulate only once in their lifetime (Fincke, 1987). Since a female may receive from one mate enough sperm to fertilize all her egg clutches, unnecessary additional copulations are energetically costly (Fincke, 1987). After copulation, females may oviposit alone or in tandem with the male (Alves-Martins et al., 2012; Corbet, 1999; Guillermo-Ferreira & Del-Claro, 2011).

Although damselflies are abundant and greatly diversified in the Neotropics, aspects of their life history, ecology and behavior are not well known (Cordero-Rivera & Stoks, 2008; Hamilton & Montgomerie, 1989). Here, we describe the reproductive behavior of a common Brazilian species, *Acanthagrion truncatum*, a widespread genus whose behavior is practically unstudied.

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The aim of this study was to characterize mating and oviposition behavior, as well as male–male interactions.

Materials and methods

Study species

Males are blue with green post-ocular spots and labrum, black mid-dorsal and humeral stripes (Figure 1) and remain perched at the water waiting for females. Females are light green, with the same black pattern as males, a ventrally yellowish abdomen and green post-ocular spots, remaining on terrestrial vegetation near the pond and approaching the water to mate and oviposit (see Leonard, 1977, for a full description of this species).

Field study

Field work was carried out in a Neotropical savanna pond in the Ecological Reserve of the Clube de Caça e Pesca Itororó de Uberlândia, Uberlândia, Brazil (CCPIU) (15°57′ S, 48°12′ W; altitude 863 m; 640 ha; see Réu and Del-Claro, 2005, for further details). The pond is square, measuring approximately 50 × 50 m. We searched for individuals on the vegetation by tapping the grass with the insect net and wading through the water, which made the animals fly a short distance and perch, making them conspicuous. We captured and marked 78 males and 46 females with a unique number in the right forewing with a hydrophobic pilot pen.

During 14 days between 26 May and 14 June 2008, we made 60 hours of observations of reproductive behavior to quantify and describe copulations and ovipositions. Thus, assuming that few individuals survive more than two weeks (no more than five days elapsed from marking to last recapture), we estimate that we recorded a significant sample of the reproductive behavior of

Figure 1. A mating couple of the damselfly Acanthagrion truncatum.
this population. Reproductive behavior was categorized following the phases described for other damselflies (Corbet, 1999; Cordero, 1989; Miller, 1987).

We made 30 hours of observations on eight days between 23 November and 4 December 2007, during which we recorded every male–male interaction, classifying each in one of the following behavioral categories: (1) chase; (2) wing or threat display (Fincke, 1987); (3) ignore; (4) approach; (5) grab; (6) hover; and (7) face off (Robertson, 1985; Sirot & Brockmann, 2001).

Results

Reproductive behavior

We recorded a total of 21 copulations and 31 oviposition events. Reproductive behavior started around 10:00 h, with the first individuals approaching the water. Males arrived at the pond and perched on *Eleocharis* sp. stems near the margins, patrolling the water and searching for females. No male was found on the surrounding vegetation.

Females remained feeding on the grassy vegetation around the pond until they flew to the pond and perched on the oviposition site without attempting to oviposit. The females remained on the same perch until a male approached. When a female was seen alone at the pond, it was observed until it met with a male (*n* = 19 females). If receptive, the female allowed the male to grab her and form the tandem (*n* = 10, 45% of events); if not, she faced him off (*n* = 5, 23% of events) or performed the wing display to ward him off (*n* = 7, 32% of events). Nine females flew away after rejecting the male and we lost them from sight. Three females were observed to reject one male and accept another male. Also, after copulation, the females sometimes resisted flight in tandem (11 of the 21 copulations observed) apparently trying to free herself from the male. No female was observed visiting the pond more than once.

When a male found a receptive female, he rushed towards her and grabbed her prothorax with his anal appendages to form the tandem position. We recorded 10 complete copulations from the 21 observed. The couple might remain a few seconds or up to 24 minutes in precopulatory tandem, while the male made invitation movements, sperm transfer (1–5 s) and precopulatory genital touches (range: 3–37 times).

The copulation *per se* consisted of (i) an initial immobile phase that lasted *ca.* one minute; (ii) stage I of copulation lasting 21.7 ± 10.28 minutes (*n* = 10), when the male moved his first two abdominal segments 15.33 ± 3.26 (*n* = 6) times per minute with short pauses and up and down movements of the entire abdomen, a behavior involved in sperm displacement (Miller & Miller, 1981); (iii) stage II of copulation lasting 1–3 minutes, when the male moves his abdomen thrusting the penis in a high frequency; and (iv) the motionless stage III lasting less than a minute, which ended the copulation.

After copulating, the couple remains in post-copulatory tandem for 10.33 ± 4.96 minutes (*n* = 12, of the 21 copulations observed) before ovipositing in tandem. During all copulatory events the male kept spreading his forewings alternately, one after the other. Of the total 46 couples observed, we observed six pairs from the beginning of copulation to the end of oviposition. From these 46 mating events, 41 males and 34 females were marked. Hence, 54.6% (41/75) of the marked males and 77.2% (34/44) of the marked females were seen copulating or in tandem oviposition. The whole copulation lasted 25.6 ± 3.26 minutes (range: 13–46).

We recorded 12 complete ovipositions from the 31 observed. Oviposition lasted 43.08 ± 22.17 minutes (range: 19–89) and females oviposited in tandem on floating grass, macrophytes stems, leaves and plant debris. The couple changed their perch 13.09 ± 7.9 times during the oviposition process and were harassed by other males that tried to disrupt the tandem (*n* = 28). Only one
male was successful in tandem disruption. While moving, the male in tandem can face off other males \((n = 5)\) or remove them from their perch \((n = 4)\).

The female may submerge up to three times when ovipositing \((n = 8)\) and remain \(11.12 \pm 10.85\) minutes underwater \((n = 16; \text{range: 1–37 min})\). Of the eight females that oviposited underwater, only one male was able to recapture the female, grabbing her back as soon as she left the water. Females that submerged normally continued to oviposit alone and no male approached. No male helped the female to emerge. The females used the vegetation to climb out of the water. The male usually perched near the underwater female and chased other males that approached. All males except one lost the location of the ovipositing female after pursuing another male. No female died while ovipositing underwater.

Copulations were concentrated between 12:00 and 14:00 h (71%, Figure 2), with ovipositions extending up to 15:00 h. By 14:00 h, the damselflies reduced their activity, leaving the pond between 15:00 and 16:00 h and hiding in the vegetation around the pond. Only one male copulated twice and no female was seen copulating more than once.

**Male–male interactions**

When two males met each other (i.e. when they met in flight or perched on the same perch), in 31% of the cases \((46/150)\) one male chased the other and in 35% \((52/150)\) they faced off against each other. Less frequently, they grabbed (5%), hovered near (14%), performed a threat display to (4%), approached (5%) or ignored (6%) another male.

**Discussion**

Male–male interactions follow a pattern also found in *Ischnura* (Robertson, 1985; Sirot & Brockmann, 2001), mainly consisting of short chases and facing off. Although males engage in pursuits, they seem to be non-territorial because they were not site attached.

Copulation duration in *A. truncatum* \((\text{ca. 25 minutes})\), although much shorter than in some coenagrionids like *Ischnura senegalensis* \((395.3\) minutes) and *Nehalennia speciosa* \((136\) minutes), is similar to others (e.g. *Coenagrion mercuriale*, 23 min; *C. puella*, 28 min) and within a range that is common in non-territorial odonates (Cordoba-Aguilar et al., 2009). Cordoba-Aguilar
et al. (2009) discuss that long copulations in damselflies are associated with sperm removal abilities.

The fact that females remain on the surrounding vegetation and visit the pond only to mate and oviposit, and that they avoid most mating attempts, suggest that female choice may exist in this species. Additionally, it seems that the only way a female may copulate more than once in her lifetime is when an ongoing copulation is interrupted. This might occur when a male steals the female or when a female emerges after underwater oviposition and is grabbed by another male.

Fincke (1986) observed that in Enallagma hageni, underwater oviposition favors male vigilance and female survival when emerging from the water. Here, we show that underwater oviposition in A. truncatum does not favor male vigilance. Underwater ovipositing females remain submerged and get out of the water by themselves, with no male help. In contrast to E. hageni, in which females are guarded and sometimes rescued from the water by males (Fincke, 1986), most male A. truncatum were not able to guard these females and lost them. Males probably attempt to guard the females to avoid losing them to other males, but fail to find them again after chasing other males away. We then suggest that underwater oviposition in A. truncatum may be a tactic adopted by females to disrupt male guarding. If we consider that in tandem oviposition may be costly to females, females should develop strategies to avoid male guarding.

Pairs of A. truncatum typically changed perch several times during oviposition. Usually, female Odonata oviposit in widespread sites to increase the chances that at least some larvae will survive (Buskirk & Sherman, 1985; Schenk et al., 2004). Females may also change perch to test the substrate with the ovipositor (Martens, 1992).

In conclusion, this study presents the first description of A. truncatum behavior, which is quite similar in most aspects to that of other Coenagrionidae. Knowledge of the reproductive behavior of Neotropical Odonata is still poor and should be stimulated.

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