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Does water intake after oviposition indicate the end of oviposition and egg depletion in Odonata females?

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Oviposition in odonate females is usually considered finished when the female leaves the oviposition site. However, considering that many species lay their eggs within a set of bouts it is difficult to distinguish between the end of the oviposition bout and the end of the oviposition episode because the females can change the oviposition site from one bout to another. In this study, post-oviposition drinking is suggested as behavioral indicator not only for the end of an oviposition episode but also for egg depletion in females, as investigated in 11 species (six zygopterans and five anisopterans) from five families. This behavior comprises water intake that the female performs at the end of oviposition by dipping the mouthparts in the water a few times before leaving the oviposition site. The role of downward bending of the abdomen displayed during water intake is also discussed.

Keywords: Odonata; oviposition; egg depletion; reproductive behavior; drinking

Introduction

Odonates have frequently been used for studies of lifetime mating success (e.g. Banks & Thompson, 1985; Bennett & Mill, 1995; Cordero, 1991; Fincke, 1982, 1986; Stoks, 2001), particularly because they are morphologically and behaviorally suitable as model insects for mark-recapture surveys (Cordero-Rivera & Stoks, 2008). Lifetime mating success is typically considered as a suitable measure for individual fitness in males (Thompson, Hassall, Lowe, & Watts, 2011). Some studies on females go one step further and assess lifetime reproductive success by measuring also the number of eggs oviposited after each mating (e.g. Bennett & Mill, 1995; Fincke, 1986).

There are several methods to estimate clutch size in odonates. One is to stimulate the female to lay her eggs (e.g. Boehms, 1971; Koch & Suhling, 2005; Śniegula & Johansson, 2010). Another way is to measure the duration of oviposition, which is easily quantified in the field, combined with prior estimations of egg deposition rate (e.g. Koenig & Albano, 1987). However, due to the fact that females of several species lay their clutch in several bouts (e.g. *Urothemis edwardsii*, Khelifa et al., 2013) or change reproductive areas during oviposition, it is difficult to determine whether a female has finished an oviposition episode because she is able to lay in different bouts at different oviposition sites (Koch & Suhling, 2005). Moreover, it is even impossible to know

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whether a female has laid all or only a fraction of her mature eggs during an oviposition episode. Dissection of some females was used as an alternative to confirm egg depletion after oviposition (Bennett & Mill, 1995; Khelifa, Zebsa, Kahalerras, & Mahdjoub, 2012). To obtain appropriate estimates of egg production and lifetime reproductive success it is crucial to know whether a reproductive female lays all her mature eggs or only part of them. Here I describe a non-lethal way to infer the end of oviposition and probably also egg depletion in odonates.

Corbet (1999) states that adult odonates meet part of their water requirements through feeding, because their food contains 60 to 80% water (Fried & May, 1983). Although drinking may be a common behavior during the entire adult lifespan of both males and females (Corbet, 1962, 1999; Wildermuth, 2008), it has received very little attention. It has been suggested that drinking is associated with activities such as bathing, which plays a role in evaporative cooling and prevents desiccation (Hutchinson, 1976; Miller, 1964; Tracy, Tracy, & Dobkin, 1979). In the present study we assess whether water intake of females after egg laying in six Zygoptera species with endophytic oviposition and five Anisoptera species exhibiting exophytic oviposition may be used as reliable indicator to the end of oviposition and probably also egg depletion.

Material and methods

Study sites

The study was conducted at four localities within the Seybouse watershed, Northeast Algeria. Beddoud (S1) (36°31′54.08″N, 7°22′48.20″E) is a 0.4 ha pond, 3 km northwest of El Fedjoudj province (Hadjoudj et al., 2014). Boumahra stream (S2) (36°27′24.50″N, 7°30′29.53″E) is a shallow watercourse with slow flowing water, about 6 km east of Guelma city. El Fedjoudj channel (S3) (36°28′21.43″N, 7°22′41.12″E) is a 300 m slow flowing and relatively shallow watercourse situated in Seybouse upstream, 5 km west of Guelma city. Old bridge canal (S4) (36°28′16.38″N, 7°22′48.57″E) is a very shallow artificial watercourse located 300 m west from El Fedjoudj channel.

Definition of technical terms

- Clutch: "complement of oocytes that mature together to produce a batch of eggs which are typically laid during an episode" Corbet (1999).
- Oviposition bout: According to Corbet (1999) "applied to oviposition, a spell of uninterrupted egg laying during an episode that may include several bouts, each separated by perching".
- Oviposition episode: According to Corbet (1999) "applied to oviposition, a single visit to the oviposition site during which egg laying may occur in several bouts, each separated by perching".
- Egg depletion: When no mature egg is left in the female abdomen.

Field observations and laboratory manipulations

Oviposition observations of 11 species of odonates (Table 1) were made on marked mature females in natural populations during their reproductive season in 2012 and 2013 from April to July. Only complete oviposition episodes were taken into account in further analysis. The termination of an oviposition episode was assumed when a female left the water, and this often means that the female has exhausted its supply of mature eggs (Corbet, 1999). To calculate the proportion of eggs left in each female's abdomen after oviposition, data on both clutch size

Table 1. Sampling sites of the 11 study species.

Suborder	Family	Species	Study site
Zygoptera	Coenagrionidae	*Coenagrion caerulescens*	S3
		Erythromma lindenii	S3
		Ischnura graellsii	S3
	Platycnemididae	*Platycnemis subdilatata*	S2, S3
	Lestidae	*Sympecma fusca*	S1
	Calopterygidae	*Calopteryx haemorrhoidalis*	S2, S3
Anisoptera	Libellulidae	*Crocothemis erythraea*	S1
		Orthetrum chrysostigma	S4
		Orthetrum coerulescens	S4
		Orthetrum nitidinerve	S4
		Sympetrum fonscolombii	S1

S1 = Beddoud, S2 = Boumahra stream, S3 = El Fedjoudj channel, S4 = old bridge canal.

before (when a female first visited the oviposition site) and after oviposition (when a female finished oviposition and drank water) were used. In one study species, *Orthetrum coerulescens*, clutch size was estimated artificially using the method described by Khelifa et al. (2012), which consists in immersing the last abdominal segments in a vial containing water. In five species (*Coenagrion caerulescens*, *Erythromma lindenii*, *Ischnura graellsii*, *Platycnemis subdilatata*, and *Sympecma fusca*) clutch size was estimated by capturing 10 females during copulation, killing them in 70% ethanol, and dissecting them in the laboratory. In five other species, i.e. *Calopteryx haemorrhoidalis*, *Orthetrum nitidinerve*, *Orthetrum chrysostigma*, *Crocothemis erythraea*, *and Sympetrum fonscolombii*, the estimated clutch size was taken from the literature. Mean clutch size of *C. haemorrhoidalis* was taken from Cordero and Andrés (2002), that of *O. nitidinerve* from Khelifa et al. (2012), and those of *C. erythraea*, *O. chrysostigma*, *and S. fonscolombii* from Koch and Suhling (2005). In order to estimate the number of mature eggs left after water intake, 10 females of each study species were captured just after performing water intake then they were dissected for egg counting. Eggs were counted with the aid of a dissecting microscope. Immature eggs were not considered; these are easily distinguished by their lack of a chorion. All studied species were regionally and locally common, thus our sampling did not have a considerable effect on the natural populations.

Statistical analyses

Statistical tests were performed using R 3.1.2 (R Core Team 2015). One-sample *t*-tests were carried out to test differences between estimated clutch size from the literature and the eggs remaining after water intake. Mann–Whitney U tests were conducted to check whether clutch size before oviposition and after water intake is different. Values are presented as mean ± SD.

Results

Females of all 11 studied species showed water intake at the end of oviposition. After drinking water females did not come back to the reproductive sites. In no species did females drink water between oviposition bouts of the same episode. The recorded frequency of water intake after oviposition varied between 0.59 in *Ischnura graellsii* and 1.0 in *Orthetrum chrysostigma* and *Sympecma fusca*, with a mean of 0.86 ± 0.13 for all species (Table 2). For water intake the females hovered above the water, then rapidly swooped down and struck the water surface with their mouthparts one to four times. While performing this behavior, in 55–91% (73 ± 11%) of

Table 2.   Number of ovipositions surveyed and details about the water intake behavior in 11 species of odonates.

Species	No. oviposition episodes surveyed	No. observations of water intake	No. observations of curved abdomen	Frequency of water intake[a]	Frequency of curved abdomen[b]
Coenagrion caerulescens	11	10	7	0.91	0.70
Erythromma lindenii	15	11	10	0.73	0.91
Ischnura graellsii	17	10	8	0.59	0.80
Platycnemis subdilatata	23	17	10	0.74	0.59
Sympecma fusca	15	15	9	1.00	0.60
Calopteryx haemorrhoidalis	34	30	23	0.88	0.77
Crocothemis erythraea	22	20	11	0.91	0.55
Orthetrum chrysostigma	13	13	10	1.00	0.77
Orthetrum coerulescens	16	13	9	0.81	0.69
Orthetrum nitidinerve	27	25	20	0.93	0.80
Sympetrum fonscolombii	12	11	9	0.92	0.82

[a]Frequency of water intake is calculated as the number of observations of water intake divided by the total number of oviposition episodes surveyed.
[b]Frequency of curved abdomen is calculated as the number of observations of abdomen curving divided by the total number of oviposition episodes surveyed.

Table 3.   Proportion of eggs left after and without water intake in 11 species of odonates.

Suborder	Species	No. of eggs after water intake	No. of eggs without water intake	Clutch size	Percentage of eggs remaining
Zygoptera	*Coenagrion caerulescens*	1.44 ± 1.42 (10)	—	177.3 ± 49.81 (10)	0.81
	Erythromma lindenii	4.00 ± 4.18 (10)	45.33 ± 12.05 (3)	476.7 ± 116.58 (10)	0.84
	Ischnura graellsii	4.77 ± 3.59 (10)	26.2 ± 2.08 (5)	207.7 ± 43.85 (10)	2.30
	Platycnemis subdilatata	1.33 ± 2.39 (10)	44.2 ± 6.00 (5)	253.2 ± 123.49 (10)	1.08
	Sympecma fusca	2.77 ± 2.58 (10)	—	210.7 ± 50.78 (10)	1.31
	Calopteryx haemorrhoidalis	2.55 ± 3.39 (10)	—	202.3 ± 10.6 (71)*	1.26
Anisoptera	*Crocothemis erythraea*	4.4 ± 5.21 (10)	—	870.5 ± 363.52 (6)*	0.51
	Orthetrum chrysostigma	7.8 ± 7.31 (10)	—	1346.63 ± 635.37 (23)*	0.58
	Orthetrum coerulescens	8.3 ± 9.23 (10)	—	–1120.38 ± 330.66 (10)	0.74
	Orthetrum nitidinerve	8.8 ± 9.28 (10)	—	2186.75 ± 722.25 (49)*	0.40
	Sympetrum fonscolombii	9.4 ± 10.17 (10)	—	633.96 ± 382.15 (41)*	1.48

Note: Asterisks indicate data that were taken from the literature: *Calopteryx haemorrhoidalis* (Cordero & Andrés, 2002); *Orthetrum nitidinerve* (Khelifa et al., 2012); *Crocothemis erythraea*, *Orthetrum chrysostigma*, *and Sympetrum fonscolombii* (Koch & Suhling, 2005). Clutch sizes before oviposition and after water intake of all species were significantly different (one-sample *t*-test: $p < 0.0001$ for species with clutch sizes taken from the literature; Mann–Whitney tests: $p < 0.0001$ for the remaining species).

cases the female's abdomen was curved downward. After water intake most zygopteran females perched next to the oviposition site or a few meters away when male density was high. After landing a droplet of water was seen attached to the females' mouthparts. In anisopterans, water droplets were recorded only in *Orthetrum nitidinerve* and *Crocothemis erythraea* because the females of the other studied species left the oviposition site without perching nearby.

Females that were dissected after having shown water intake carried 5.05 ± 3.02 eggs, which corresponds to 1.03 ± 0.55% of the total clutch size. The percentage of eggs left varied between 0.4% in *O. nitidinerve* and 2.29% in *I. graellsii* (Table 3). Due to the high frequency of water intake after the surveyed oviposition episodes, only a few females from three species, i.e. *Erythromma lindenii*, *I. graellsii* and *Platycnemis subdilatata*, were captured to estimate the number of eggs left without drinking. The number of eggs found in these non-drinkers was 11.33, 5.49 and 33.23 times larger than that that recorded in drinkers. Moreover, when we pool all three species together the number of eggs carried represents is 13.19 ± 4.0% of the total number of eggs.

Discussion

From the results of this study it is inferred that water intake in odonate females can be used as an indicator of the end of an oviposition episode and probably egg depletion. The observation of water intake after oviposition in several endophytic zygopterans and exophytic anisopterans belonging to five families indicates that water intake at the end of oviposition may be an universal feature in odonates, thus being considered an integral component of an individual's oviposition episode.

Even though the sample size of females that did not drink after oviposition was too small to perform statistical tests, the large difference between the two supports our hypothesis. Females that did not drink water probably stopped oviposition due to disturbance or decided to change reproductive area.

The fact that females engage in this behavior suggests that that odonates may not rely on food alone to obtain sufficient liquid for their entire adult lifespan, but they also have to drink water by dipping their mouthparts in the water, as was noted by Hutchinson (1976). In fact, it is reasonable to suggest that from emergence up to maturation individuals rely only on food to meet their water requirements because they generally spend most of their time away from the water (Stoks, 2001). During this study, mature females were observed to drink at reproductive water bodies, which indicates that food alone is probably not enough to obtain ample liquid. In the studied species oviposition was performed mainly during the hottest time of the day, and it is a time and energy consuming behavior during adult life. Water loss during oviposition can be substantial, thus females have to take an amount of water just after finishing egg laying to compensate for dehydration (Tracy et al., 1979). Similarly to studies on mosquitoes which have shown that water stress reduces egg production (Benoit, Lopez-Martinez, Phillips, Patrick, & Denlinger, 2010), it is probable that dehydration has a negative effect on individual foraging and reproductive abilities in odonates.

I assume that the frequency of water intake depends mainly on local temperature, that is, during hot days and in hot areas like North Africa females suffer substantial water loss during oviposition and therefore need water to avoid dehydration (Tracy et al., 1979). The investigation of the frequency of drinking behavior in odonates living in cool temperate zones where water loss is low might reveal whether liquid intake after oviposition is dependent on water loss or is obligatory to produce a new clutch.

I expect that Odonata females, especially those that forage far from water, drink water only after oviposition. After maturation females usually come to the water only to lay eggs, and then they return to terrestrial habitats (Stoks, 2001). Therefore, females may have only two predictable opportunities to gain water: (1) during their first arrival at the water, and (2) before departure to terrestrial areas. Considering that females of most odonates are seized by males as soon as they appear at the oviposition sites, drinking water before leaving the reproductive site is probably the best moment.

As shown in this study, water intake of females after oviposition is a reliable indicator of the fact that virtually all eggs were laid. The dissection of females of 11 species showed that no or only a very low number of eggs are left after water intake. In addition, this behavior was not observed between oviposition bouts of the same day. Therefore, odonatologists could take drinking behavior into account when they try to make an appropriate estimate of the daily duration of an oviposition episode.

Moreover, even though the downward curved abdomen during water intake was associated with bathing behavior (Hutchinson, 1976) it is also plausible to interpret it as refusal behavior towards male molesting. Indeed, abdomen curving belongs to the refusal behaviors that females usually display towards males to express reproductive rejection (e.g. Rowe, 1978; Rüppell & Hilfert-Rüppell, 2014). The fact that this behavior is expressed at a time when the female

has finished oviposition and no longer carries eggs strengthens the hypothesis that curving the abdomen downward while dipping in the water with the mouthparts might serve both as bathing and reproductive refusal towards harassing males.

Taking into account that water intake will help to estimate the number of eggs laid per visit of females ovipositing naturally, by regularly repeating this estimation on marked individuals, reliable estimates of lifetime reproductive success of both females and males could also be calculated in natural populations. This study does not cover one important aspect of Odonata oviposition which is laying a single clutch over a span of several days because the studied species did not show such an oviposition mode. Further studies should investigate water intake in such species to understand how hydration is related to egg production.

Acknowledgments

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