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Clutch size and egg production in *Orthetrum nitidinerve* Selys, 1841 (Anisoptera: Libellulidae): effect of body size and age

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Clutch size is an important fitness component often quantified artificially by inducing oviposition in libellid females. Female behavior and egg production of the yellow-veined skimmer, *Orthetrum nitidinerve*, were studied in northeast Algeria during its reproductive season. Data on reproductive behavior and biology of this Mediterranean endemic species has not been published previously. Males guarded territories within the wetland while females came only to lay their eggs and then went back to terrestrial habitat. In this study we induced oviposition, which depletes all the female eggs, to obtain estimations of egg deposition rate and subsequently clutch size. On average an induced clutch was ca. 2200 eggs while a natural one was about 970 eggs. Artificial clutches were positively correlated to body length but negatively related to mature lifespan. The rate of egg deposition was higher in the afternoon than in the morning, probably because of differences in temperature. During their mature lifespan females oviposited between one and three artificial clutches.

Keywords: clutch size; induced-egg laying; egg production; oviposition; *Orthetrum nitidinerve*; dragonfly; Odonata

Introduction

During the breeding season of odonates, mature females with higher foraging efficiency produce more eggs and thus usually have higher fecundity (Richardson & Baker, 1997). Clutch size is an important reproductive component, subject to large inter- and intraspecific variability. Within the same species body size, age, and parasitism are known to influence the number of eggs produced by a female over her lifetime (Corbet, 1999).

In the field estimation of clutch size is relatively easier in endophytic species; by collecting the plant tissue with eggs their numbers can easily be counted afterwards (Bennett & Mill, 1995; Fincke, 1986). In exophytic odonates where eggs are usually spread on the water surface, females were induced artificially to lay their eggs (Boehms, 1971; Convey, 1989; Hottenbacher & Koch, 2006; Koch & Suhling, 2005; Lempert, 1987; McVey, 1984; Sahlén & Suhling, 2002; Schenk & Söndgerath, 2005; Schenk et al., 2004). In both cases, the number of eggs deposited was subsequently estimated based on egg deposition rates and oviposition durations. However, using

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data from induced egg oviposition to estimate naturally laid clutch size could be subject to more or less systematic error depending on oviposition behavior (M.L. May, personal communication, 2011). In fact, many libellulids lay their eggs by dipping the distal part of their abdomen on the water surface many times. The number of eggs per dip laid by such females might substantially vary from one dip to another, which could produce under- or overestimations of clutch size. Other libellulids, on the other hand, immerse their abdomen in the water and remain immobile while continuously laying their eggs. In this case estimates of naturally laid clutch sizes are usually more accurate.

In this study we chose *Orthetrum nitidinerve* Selys, 1841, as a model species. Females of this territorial dragonfly exophytically oviposit homogeneous strings of eggs in the same laying point and are guarded (non-contact post-copulatory guarding) by a male (Khelifa et al., submitted). We estimated female egg deposition rate and clutch size, and investigated two potential covariates, namely body size and longevity.

Methods

This study was carried out in the Old Bridge canal, upstream of the Seybouse River in northeast Algeria (36°28' N, 7°22' E) (Figure 1). This canal, about 450 m long, had shallow and slow running water. The main vegetation cover was *Nerium oleander*, *Typha angustifolia*, *Juncus maritimus*, and *Paspallum distichum*. In addition to two congeneric species (*Orthetrum chrysostigma* Burmeister, 1839 and *O. coerulescens anceps* Fabricius, 1798), the zygopterans *Calopteryx haemorrhoidalis*, *Ischnura graellsii*, *Platycnemis subdilatata*, *Ceriagrion tenellum* and *Coenagrion caeruleum* also coexisted with the study species.

We chose a transect of 150 m at the lower part of the canal where the species was concentrated (Figure 1). Along the watercourse emergence (exuviae) was only observed there. We know that only a negligible number of adults might be encountered upstream since two researchers who daily surveyed a large population of *Calopteryx haemorrhoidalis* informed us that the species occurrence was very rare (only one or two males). In addition, we do not think that females

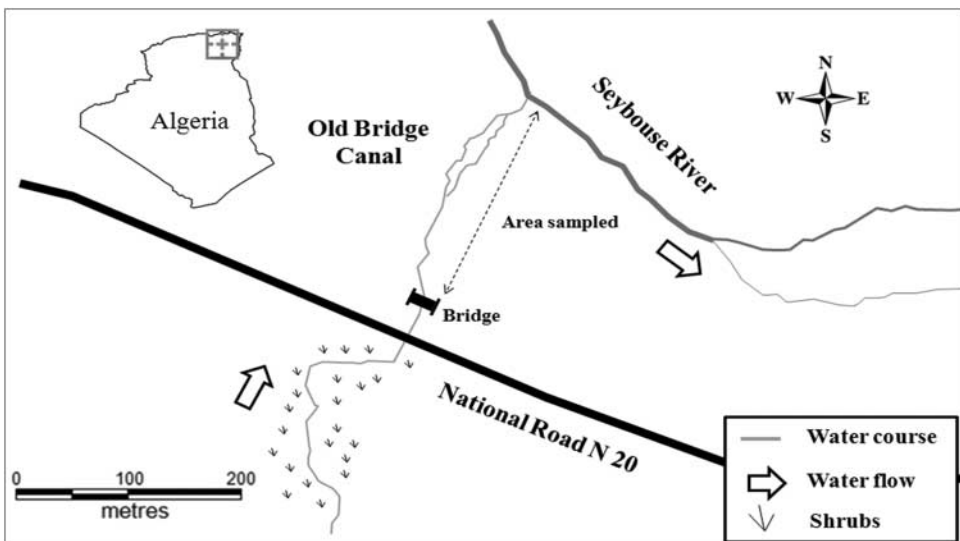


Figure 1. Location map of the sampling area in the Old Bridge canal.



Figure 2. Induced egg laying procedure in a female *O. nitidinerve*.

dispersed to another area to lay their eggs because the closest water body was the Seybouse River with fast flowing water, which is not the species' preferred habitat.

We counted adult individuals (males and females) daily at 12:00 by walking along the transect from 12 June to 11 August. We chose this hour because the peak number of reproductive pairs occurs mainly around noon.

At the study site, the reproductive season started in mid June and ended in early August. We monitored oviposition frequency and clutch size as closely as possible from 26 June to 11 August. We attempted to capture the maximum number of reproductive pairs but probably missed a few reproductive pairs at the beginning of the season, even though copulation and oviposition events were rarely observed at that time.

Sampling was divided into two main periods within a day, morning (08:00–12:00) and afternoon (12.30–17:00). Usually, no individual was seen before 09:00 or after 16:00. For several reasons we could not count the total number of eggs laid by females during natural oviposition bouts. The species produces large clutches that can contain up to thousands of eggs, which are hard to collect because they are laid within highly adhesive strings deposited against a substrate. Moreover, egg masses form a gelatinous sphere after a couple of hours, making estimation of egg numbers difficult or impossible.

We marked 30 females on the left hind wing and thorax with white 780 Edding® markers in terrestrial habitats near the canal in order to survey the duration of natural egg laying and estimate natural clutch size. It is important to note that the number of females marked and recovered ($n = 18$) for natural egg laying was small, so data obtained could only be used for comparison with induced egg laying. Similarly, we interrupted copulations and marked reproductive pairs in the canal with red permanent markers and measured the body and hind wing length with a digital caliper to the nearest 0.01 mm. Females caught in copulation were induced to lay their eggs by holding them by their wings and immersing the distal abdomen segments in a plastic container of water (Boehms, 1971) (Figure 2). Since water temperature has a strong effect on egg release rate (McVey, 1984), we kept containers in a shaded area and we used water collected from the canal. A female usually began to lay her eggs as soon as the abdomen touched the water and rarely before. Females were released after oviposition. We are confident that the process did not affect their survival since most of them were observed flying and copulating afterwards. To investigate whether females laid their entire clutch during this induced oviposition procedure,

we instantly killed 10 females from another locality by 90% ethanol just after induced oviposition, dissected them in the laboratory, and counted the remaining eggs (McVey, 1984). The maximum number of eggs counted was twelve ($\ll 0.5\%$ of the total clutch), which indicates that the procedure gave good estimates of the total number of eggs carried by a female (full clutch size).

To estimate egg deposition rate during the induced oviposition procedure, we touched the end of the female abdomen against the container edge at given time intervals so that egg strings were broken to smaller pieces. For each female performing induced egg laying, we counted the number of eggs in a string laid in five seconds and calculated the rate of egg deposition (number of eggs per second). This apparently did not affect the rate of egg deposition because females continually laid eggs nonstop until their depletion. On 10 occasions, we confirmed that this rate was quite constant through oviposition duration by repeating the previous method twice for the first and second minute (paired sample *t*-test: $t = 0.83$; $df = 9$; $p = 0.42$). We then estimated artificial clutch size as the product of egg deposition rate and total oviposition duration.

Air temperature data recorded each hour from 09:00 to 16:00 were obtained from a meteorological station situated at 9 km from the study site to document and compare temperature variation in the morning and in the afternoon and also to investigate its effect on clutch size.

Statistical analyses

All statistical analyses were performed with SPSS 17.0. Mann–Whitney tests were used to compare clutch sizes and mean air temperature recorded in the morning and in the afternoon. We carried out paired sample *t*-tests to compare between the number of eggs laid by females during the first and second minute. Spearman's correlations were calculated to estimate relationships between air temperature, body measurements, clutch size, and egg deposition rate, and between body measurements, observed mature lifespan and clutch size. Values are presented as mean \pm SD.

Results

Sex ratio

The sex ratio at the mating area along the canal was always highly male biased during our study period (Figure 3) with a daily average of 91% males. After 28 July, the number of individuals dramatically decreased, probably because of bad weather conditions recorded that day. Males were always the first to arrive at the wetland (usually at 09:00) followed by females (at 10:00). Some days, no females were observed along the whole transect. Moreover, mature females were rarely recorded alone in the canal, but mostly in tandem or guarded by a male. Females were usually observed in terrestrial habitat after oviposition and came to the wetland only to lay their eggs.

Rate of egg deposition

The rate of egg deposition was significantly different between the two sample periods (Mann–Whitney *U* test: $U = 79$, $p < 0.001$, $n = 49$) and it was positively correlated to air temperature ($r = 0.62$, $p < 0.0001$). Because of the difference in air temperature between the two sample periods ($U = 163.5$, $p < 0.0001$, $n = 33$) (Table 1), this rate was faster in the afternoon (8.37 ± 0.76 egg/s, $n = 21$) than in the morning (7.19 ± 0.76 eggs/s, $n = 28$). The rate of egg deposition

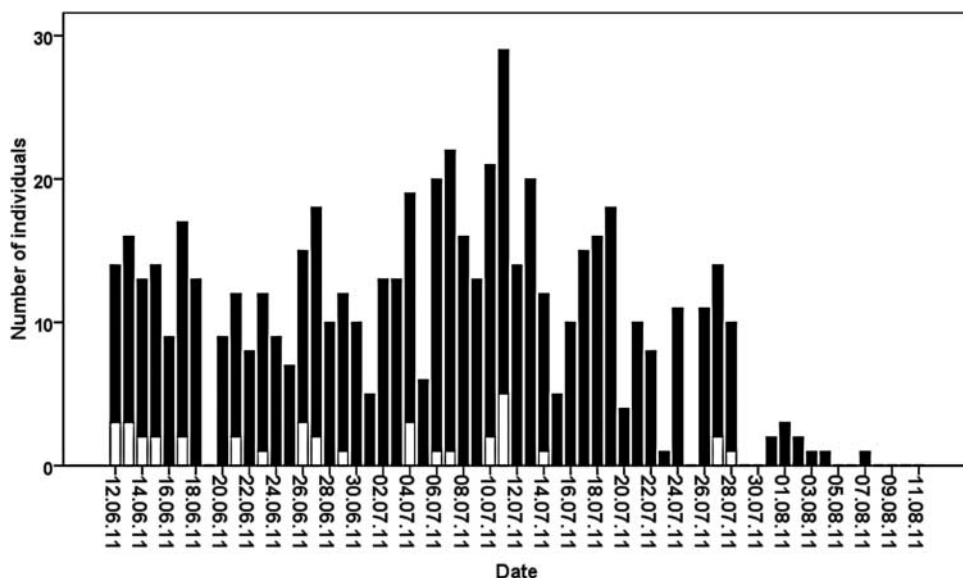


Figure 3. Daily numbers of male (filled bars) and female (open bars) *O. nitidinerve* in Old Bridge canal; on 19 June, 24, 28, and 29 July, no individuals were observed because of bad weather.

Table 1. Mean, minimum, and maximum air temperatures ($^{\circ}\text{C}$) during the study period. Data are means \pm 1 standard deviation.

	Morning	Afternoon
Mean	33.1 \pm 2	36.3 \pm 2.3
Minimum	29.9 \pm 2.1	34.6 \pm 2.5
Maximum	36 \pm 2.1	37.9 \pm 2.1

was not correlated to either body measurement (body length: $r = -0.05$, $p = 0.72$; hind wing length: $r = -0.12$, $p = 0.35$).

Clutch size

Figure 4 presents the frequency distribution of the number of induced clutches deposited by females during their mature lifespan. A total of 55 different copulation events were observed but we were only able to capture and induce oviposition of 49 breeding females. Mean artificial clutch size of *O. nitidinerve* was 2186.75 ± 722.25 eggs ($n = 49$). The number of eggs deposited was significantly higher in the morning (2490.31 ± 732.10 eggs, $n = 28$) than in the afternoon (1782.02 ± 476.86 eggs, $n = 21$) ($U = 116$, $p = 0.0003$). Since time of day had an effect on clutch size, we selected only data of females laying before noon to reveal potential relationships with selected covariates. We found a negative correlation between clutch size and observed mature lifespan ($r = -0.43$, $p = 0.02$), a positive correlation with body length ($r = 0.47$, $p = 0.01$) but no relationship with hind wing length ($r = 0.20$, $p = 0.29$). Clutch size also declined with clutch number ($r = -0.36$; $p = 0.01$, $n = 49$), but this last analysis includes clutches from both morning and afternoon, so the correlation may be confounded with the effect of time of day.

From the 30 females marked in terrestrial habitat, only 12 (40%) were recovered at the canal and these were observed during 18 natural oviposition bouts. Naturally laid clutches were significantly smaller than induced ones ($U = 4$, $p < 0.0001$). The overall mean of natural clutches was

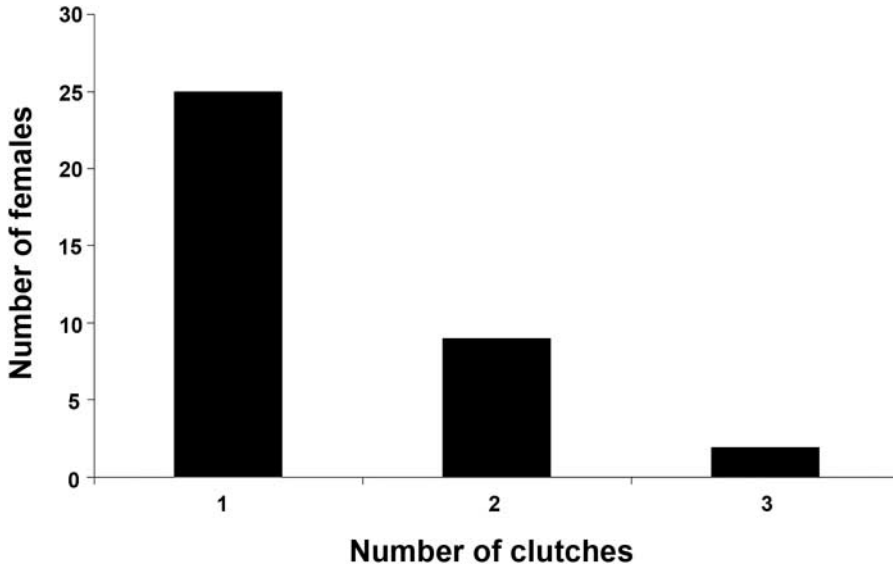


Figure 4. Frequency distribution of the number of induced clutches deposited during the study period. Average clutch size, including both morning and afternoon clutches, declined with clutch number, as follows: first clutches – 2329.01 ± 740 ($n = 36$), second clutches – 1888.67 ± 496.97 ($n = 11$), third clutches – 1265.5 ± 2.12 ($n = 2$).

974.41 ± 144.22 eggs ($n = 18$). Females that oviposited artificially were never observed copulating during the same day after the operation, but on one occasion we noted a female ovipositing naturally twice in a day. Mean observed mature lifespan of females laying induced clutches was 2.55 ± 3.02 days (range 1–12 days, $n = 49$).

Discussion

In this study we calculated clutch size by inducing artificial oviposition of female *Orthetrum nitidinerve*, a Mediterranean endemic species that has never been studied. We found that this large dragonfly produced thousands of eggs per artificial clutch and that this number depended on body size and age.

Our data indicate that *Orthetrum nitidinerve* females, like many territorial odonates, laid their eggs and soon left the water body until the next oviposition. This is thought to be the result of sexual conflict. Since males try to maximize their fitness by increasing copulation rate in the wetland, females evolved a back and forth dispersal behavior to terrestrial habitat in order to minimize their time spent in the wetland and by doing so to avoid male harassment. Male harassment may be costly in terms of lowered foraging returns, hence may reduce female condition (review in Stoks & Cordoba-Aguilar, 2012) and may also cause damage. In the study site, harassment was especially high since males of two other congeneric species (*O. chrysostigma* and sometimes *O. coerulescens*) also reacted to *O. nitidinerve* females (Khelifa et al., submitted). The consequence of the female dispersal behavior is a highly male-biased operational sex ratio in the wetland, as found in many adult odonates (Corbet, 1999; Stoks, 2001).

One finding of this study was that egg deposition rates were positively correlated to air temperature and they were higher in the afternoon than in the morning. In fact, in many insects egg deposition rate is dependent on ambient temperature (Björkman et al., 2011; Mack & Backman, 1984; Wermelinger & Seifert, 1999). In dragonflies (libellulids), McVey (1984) showed that the higher the female's abdomen temperature, specifically that of the last few abdominal segments,

which depends strongly on the temperature of the water into which she oviposits, the faster the egg release rate. The same finding was observed in *Enallagma hageni* (Zygoptera), a species that oviposits endophytically, often under water (Fincke, 1985). The rate of egg deposition of *O. nitidinerve* was similar to that estimated artificially in *Erythemis simplicicollis* (8.0 eggs/s) and *Sympetrum vicinum* (7.3 eggs/s) (McVey, 1984).

Corbet (1999) stated that factors such as body size and age could generate variations in clutch size within the same species. In this study we found an age-dependent decrease in clutch size, also observed in many zygopterans (Cordero, 1991; Gribbin & Thompson, 1990; Rowe, 1987; Watanabe & Adachi, 1987; Watanabe & Ohsawa, 1984) in which the maximum number of eggs is achieved at maturation but tended to decrease afterwards with age. This may be explained in part by the reduction of clutch size in second and third clutches (Figure 4), but the relative roles of clutch number and longevity *per se* needs further study. Clutch size, on the other hand, was positively correlated to body length (an accurate indicator of body size), as reported in many species of odonates (Sokolovska et al., 2000).

Females laid a mean of 2200 eggs per artificial clutch, comparable to the estimated number of eggs per episode laid by *O. triangulare* (Higashi & Watanabe, 1993). Clutch sizes were significantly larger in the morning than in the afternoon. Higashi and Watanabe (1993) found the same pattern in three congeneric species in Japan (*O. japonicum*, *O. albistylum* and *O. triangulare*), suggesting that females usually lay their eggs around noon. In the current study, we probably missed a few copulations at this period (mainly between 12.00 and 12.30) even though sampling efforts were high, and this made a decreasing trend in the number of eggs laid during the day. However, we found that natural clutches oviposited per visit (974.41 ± 144.22 eggs, $n = 18$) represented only 44% of induced clutches, which means that females laid naturally just a fraction of their eggs during each visit. This natural clutch size is similar to that reported for *O. cancellatum* (919 eggs, $n = 40$; Siva-Jothy, 1987) but smaller than that estimated for *O. chrysostigma* (1346.63 ± 635.37 eggs, $n = 23$; Koch & Suhling, 2005). Comparable clutches were also noted in other libellulids such as *Trithemis annulata* (924.2 ± 801.44 eggs, $n = 5$) and *Pantala flavescens* (981.65 ± 670.33 eggs, $n = 23$) (Koch & Suhling, 2005).

Female libellulids need a few days to a few weeks to mature, often far from reproductive sites (Corbet, 1999). In this study, mature females were also rarely observed at the water body because they spent the majority of their time far from the water and thus could not be observed. This resulted in an observed mature lifespan of 2.55 days.

Knowledge of species egg production is essential since the number of eggs represents the initial population size after hatching. Prior to this study no data were available on reproductive behavior or egg production of this Mediterranean endemic species. Even though *O. nitidinerve* is listed as least concern in the IUCN Red List (Riservato et al., 2009), a lack of data on its biology and ecology over its range could be problematic for its conservation.

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