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Aspects of reproductive biology and behaviour of the regional critically endangered *Urothemis edwardsii* (Odonata: Libellulidae) on Lake Bleu (Algeria)

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A study on the reproductive biology and behaviour of the regional critically endangered *Urothemis edwardsii* Selys was conducted in the relict subpopulation of Lake Bleu (North-east Algeria) during the reproductive season of 2012. The reproductive behaviour was described from the pair formation to the end of oviposition. Copulation duration was 98.55 ± 16.48 s, and the whole oviposition episode lasted 220.89 ± 32.08 s with usually three bouts interrupted by three rest periods. The species displayed a particular oviposition behaviour characterized by an alternation of contact (during the first bout) and non-contact guarding. The induced clutch size was 646.33 ± 173.10 eggs. In the laboratory, eggs showed direct embryonic development and synchronous egg hatching within the modal period of 10 days ranging between nine and 24 days. The overall hatching success was 85.39%, the main causes of mortality being infertility and unhatchability.

Reliktinės laumžirgio *Urothemis edwardsii* Selys subpopuliacijos dauginimosi biologijos ir elgsenos tyrimai Bleu ežere (Šiaurės Rytų Alžyras) buvo atlikti dauginimosi sezono metu 2012 metais. Tirtai rūšiai šiame regione gresia itin didelis išnykimo pavojus. Dauginimosi elgsenos tyrimas apėmė etapus nuo poravimosi iki kiaušinėlių dėjimo pabaigos. Kopuliacijos trukmė buvo 98,55 ± 16,48 sekundės. Visas kiaušinėlių dėjimo procesas (trys etapai ir trys pauzės poilsiui) truko 220,89 ± 32,08 sekundžių. *Urothemis edwardsii* kiaušinėlių dėjimo elgsenai būdinga kontaktinė (per pirmąjį etapą) ir nekontaktinė apsauga. Vidutinis dėties dydis buvo 646,33 ± 173,10 kiaušinėlių. Laboratorijoje embrionai vystėsi iš karto, lervos ritosi iš kiaušinėlių sinchroniškai. Išsiritimas truko 9–24 (vidutiniškai 10) dienų, išsirito 85,39% kiaušinėlių.

Keywords: odonate; dragonfly; threatened; *Urothemis edwardsii*; oviposition; embryonic development

Introduction

Urothemis edwardsii (Selys 1849) is probably the most threatened dragonfly in North Africa (Samraoui et al. 2010) as well as in the Mediterranean basin (Riservato et al. 2009) with only a single small population currently restricted to north-east Algeria. This species of tropical origin (central and southern Africa) exists in North Africa as relict in areas where environmental conditions are similar to tropical ones. Historically, subpopulations existed regionally in Algeria, Tunisia, Israel, Palestine and Jordan (Suhling and Clausnitzer 2009). However, records show that over the last two decades almost all of them have become extinct due to anthropogenic pressure (Riservato et al. 2009). In Algeria, where three localities of *U. edwardsii* were known, Samraoui et al. (1993) confirmed the local extinction of two subpopulations on Lake Oubeira and Lake Noir in 1992, while Riservato et al. (2009) have recently assumed that the third subpopulation existing on Lake Bleu have disappeared as well (Appendix 1). However, subsequent visits to the latter site showed that the local population still exists with substantial numbers.

The subspecies (*U. edwardsii hulae*) of the currently extinct relict population of Jordan Valley (Lake Hula) was described by Dumont (1975) based on the small extent of hindwing basal spot. Riservato et al. (2009) proposed that this subspecies distinction was not valid because it could be only a phenotypic variation of nominotypical populations. In the assessment of the status and distribution of the Mediterranean Basin odonates, Riservato et al. (2009) reviewed the available information on *U. edwardsii* and highlighted many gaps in the knowledge of its biology, ecology and conservation measures. The current paper presents some aspects of the reproductive biology and behaviour of the species on Lake Bleu.

Materials and methods

Lake Bleu (36°54′33.75″N, 8°20′17.53″E) is located in the north-west National Park of El Kala, 0.6 km north-east

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of Lake Mellah and 0.6 km south of the Mediterranean Sea. It is a 2 ha pond with half of its area covered with White Waterlilies (*Nymphaea alba*) surrounded by bank vegetation. The latter consists of dense shrubs of the Mediterranean Willow (*Salix pedicellata*) and some Common Alders (*Alnus glutinosa*) in the south-east, a line of Yellow Flag Irises (*Iris pseudacorus*) mainly in the south, a belt representing an association of the Common Tule (*Scirpus lacustris*), Narrow-leaved Cattail (*Typha angustifolia*), Cell Grown (*Lythrum salicaria*), and finally stands of Reeds (*Phragmites australis*) with tufts of Sedges (*Cladium mariscus*) at the edge of the White Waterlilies at a depth of about 1 m.

Reproductive behaviour survey

During the reproductive season of 2012, five visits were made to Lake Bleu between July and early August. Two observers surveyed the reproductive behaviour of adults from 8:00 am until 4:00 pm in the south and south-east of the waterbody where reproduction mostly occurred. Adult marking was not conducted to avoid potential damage that could have a negative impact on the critically endangered population. Different reproductive episodes from pair formation to the end of oviposition were observed. As the species did not leave water after an oviposition bout without disturbance, reproductive pairs were easily surveyed during the whole reproductive episode. When confusion between different individuals occurred due to the interference of mature males, data were not recorded. However, those cases were rare. Copulation and oviposition durations were recorded to the nearest second. The number of dips taken by females during the whole oviposition episode as well as the number of bouts and rest periods was documented.

Clutch size and embryonic development

Only seven females were captured after copulation and induced artificially to lay their eggs in plastic vials half-filled with water (Khelifa et al. 2012). A few hours later, the number of eggs was counted to determine the clutch size, and the length and width of 10 eggs from a single clutch were measured to the nearest 0.01 mm using a dissecting microscope. Each clutch was placed in a vial ($10 \times 5 \times 4\,cm^3$) under natural light conditions. Air temperature in the laboratory was taken three times a day (at 9:00 am, 2:00 pm and 7:00 pm) daily with an electronic thermometer to the nearest 0.1 °C. Water was renewed three times each week before hatching and daily after hatching. Eggs were checked for hatching daily. Prolarvae were isolated from the original vial and placed in another one. They were counted and then translocated to another site as part of another study. Egg hatching was considered to have finished when no prolarvae were found after 10 consecutive days of checking. Values presented hereafter are mean ± SD.

Results

Reproductive behaviour

Adult males waiting for females began to appear approximately between 8:45 and 9:00 am. They were observed to hold a support for females to perch a few metres above water. It was usually sticks standing in an open area, where there was no vegetation or it was not dense and high, that served as supports. Grazed tufts of vegetation were also guarded by males. However, dragonflies showed a marked preference for metal bars of barrier fences used for bordering local residential domains. A single male was usually observed on top of an 80–100 cm high support holding an area within a radius of about 1.5–2 m.

Males intercepted females in the air (pair formation). They tended to perch on high supports like reeds (*Phragmites australis*), especially when disturbed by other males. They proceeded with wheel formation as soon as they perched, although some pairs had difficulties in this process at their first attempt. Copulation duration was 98.55 ± 16.48 s ($N = 10$). After that, the male grabbed the female and headed towards oviposition sites, which were characterized by open shallow water with emergent aquatic plants (*Myriophyllum spicatum*) and a few stands of vegetation (*Iris pseudacorus*). The pair hovered about 10–20 cm above the water surface and took several dips for about 1 min 30 s (tandem oviposition); then, the pair broke up and the female went to perch and rest on the closest support (about 50 cm in height) for approximately half a minute. The male made a few patrols around the oviposition site and then perched next to the female. After resting, the female separated from the male, hovered above water and took some dips for more than 1 min. She was guarded by the male, who was flying around her, chasing off other contestant males. When the latter ones were gone, the male would sometimes perch next to the female. The female perched for the second time to rest for about 20 s. After that she oviposited for nearly 30 s, then perched for the third time for five seconds and went away from water. There was no other wheel formation after the separation of the pair. Also, a remarkable decreasing trend in dip frequency was observed from one bout to another within the same oviposition episode. The second visit of females to reproductive sites was not confirmed because individuals were not marked. The whole oviposition episode lasted for 220.89 ± 32.08 s ($N = 9$) with an average of 55.67 ± 4.83 ($N = 6$) dips, four bouts and three periods of rest shorter than 30 s.

Clutch size and egg hatching duration

As females that were collected in the afternoon might have laid some eggs earlier in the morning, it was considered that only those induced to lay their eggs in the morning could produce a full clutch size. The mean artificial clutch size of *U. edwardsii* was 646.33 ± 173.10

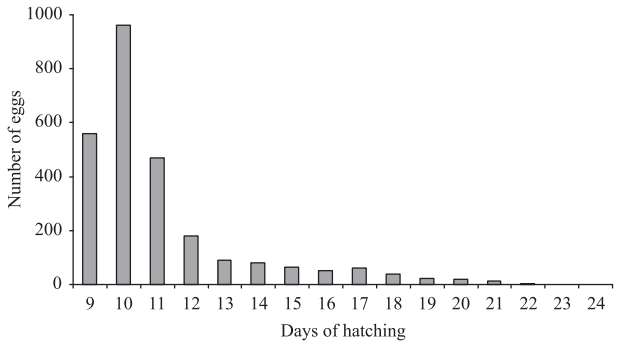

Figure 1. Frequency distribution of *Urothemis edwardsii* (Selys 1849) (Odonata: Libellulidae) hatching dates under laboratory conditions. The total number of hatched eggs was 2508. Laboratory temperature was 27.5 ± 1.02 °C.

eggs ($N = 3$) with a maximum of 784 eggs. An egg was 0.54 ± 0.01 mm (0.52–0.55 mm, $N = 10$) in length and 0.43 ± 0.01 mm (0.40–0.45 mm, $N = 10$) in width. In the laboratory, air temperature varied within and between days showing a mean of 27.5 ± 1.02 °C ranging between 25.9 and 28.5 °C. The hatching time ranged from nine to 24 days with the modal period of 10 days (Figure 1). A total of 2937 eggs were collected (all clutches included) and only 2508 eggs hatched resulting in an overall hatching success of 85.39%. The mean hatching success between different clutches was 82.29 ± 9.37%. Infertility (3.74%) and unhatchability (10.96%) probably due to fungi were the two causes of egg mortality.

The hatching time of 78.11% of eggs, which hatched within 9–11 days of oviposition, was well synchronous (considering only those that hatched). The distribution of hatching dates presented in Figure 1 shows a positive skewness, because a small proportion of eggs lasted 24 days before hatching.

Discussion

Many aspects of the biology, ecology and behaviour of the *U. edwardsii* that are essential for the identification of species ecological requirements and overcoming threats facing natural populations have never been studied in any region of its global distribution. Due to the lack of the above-mentioned knowledge, many relict subpopulations have become extinct along the South Mediterranean (Riservato et al. 2009). This study fills in some gaps in the knowledge of the biology and reproductive behaviour of the species.

The reproductive behaviour of *U. edwardsii* was quite similar to many territorial Palearctic libellulids (Corbet 1999) except for oviposition. The latter's behaviour was quite complex, presenting an alternation of contact (*Sympetrum* type) and non-contact guarding (*Orthetrum* type) within the same reproductive episode. This kind of oviposition is rare in odonates and is known from species of *Tramea* (Corbet 1999); however, *Tramea*-type guarding involves frequent wheel formation after the female solitarily dips water (as was noted in *Tramea transmarina euryale* (Sakagami et al. 1974)) and this was not observed in *U. edwardsii*. According to literature, the oviposition behaviour of *U. edwardsii* described here represents a new oviposition behavioural sequence that has never been reported. Our observations on reproductive behaviour are limited and need further studies based on marked individuals to determine the species' ethogram and factors influencing individual behavioural decisions.

The induced clutch size of *U. edwardsii* (646.33 ± 173.10 eggs) was similar to that of *Sympetrum fonscolombii* (633.96 ± 382.15 eggs), slightly smaller than that of *Trithemis kirbyi* (734.58 ± 488.31 eggs) (Koch and Suhling 2005) and *Orthetrum triangulare* (700 eggs) (Watanabe and Higashi 1993), but substantially smaller than that of many other libellulids (see Corbet 1999; Koch and Suhling 2005). However, the clutch size is known to be negatively correlated to egg size (Sahlén and Suhling 2002). With the egg length of 0.52–0.55 mm and the egg width of 0.40–0.45 mm, *U. edwardsii* had larger eggs than many other libellulids (Corbet 1999; Sahlén and Suhling 2002; Koch and Suhling 2005), and it also fell within the egg size range of *Sympetrum fonscolombii* (length: 0.48–0.57 mm, width: 0.33–0.44 mm) (Sahlén and Suhling 2002).

U. edwardsii showed a marked synchrony in egg hatching under a laboratory temperature of 27.5 ± 1.02 °C (25.9–28.5 °C) with 78% of eggs hatching within 9–11 days, while the other 22% of eggs took up to 24 days to complete embryonic development and hatch. At a slightly higher temperature (27–32 °C), the minimum duration of egg hatching of *U. assignata* was 6–7 days (Hassan 1977). Such a short embryonic development is widespread in odonates of tropical origins, and especially those inhabiting temporary water bodies (Corbet 1999). Positive skewness of the temporal distribution of egg hatching observed in *U. edwardsii* is also the usual pattern for odonates with direct embryonic development (Corbet 1999), although most studies referred to the minimum hatching duration and did not survey the whole temporal pattern. Such a survey was conducted on the temperate Zygopteran *Pyrrhosoma nymphula* by Bennett and Mill (1995), who found that egg hatching ranged between 22 and 56 days with about 60% of eggs hatching within 24 and 26 days.

Although water in vials where eggs were placed was renewed frequently, in some clutches fungi usually occurred after two weeks. A small proportion of eggs was identified as infertile since their colouration remained white-yellowish and did not turn brown as that of the majority of eggs. This was probably due to the artificial egg laying, which is known to negatively affect the fertility of eggs (Corbet 1999). Although laboratory studies have shown that ambient temperature may affect egg mortality (Waringer and Humpesch 1984; Lutz and Rogers 1991), variability in the air temperature recorded during this study was not significant enough to induce mortality of *U. edwardsii* eggs.

In conclusion, this study provides an essential background to the reproductive behaviour and biology of

the regional critically endangered *U. edwardsii*. Data presented here could be used for developing new conservation strategies aimed at upgrading the species' status in Algeria as well as in the other regions.

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Appendix 1. The global distribution range of *Urothemis edwardsii*. The question mark in Tunisia means that no evidence of the population was recorded there.