African Journal of Ecology

# Partial bivoltinism and emergence patterns in the North African endemic damselfly *Calopteryx exul*: conservation implications

Rassim Khelifa[1,2]*

[1]*Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland,* [2]*Faculté des sciences biologiques et agronomiques, Département de biologie, Université de Mouloud Mammeri, 15000 Tizi Ouzou, Algérie*

## Abstract

*Calopteryx exul* is an endemic endangered damselfly that suffers considerable habitat degradation and local extinctions throughout its geographic range. Although recent studies have investigated its distribution, ecology and larval systematics, the life history of the species is still unknown. In this study, a field survey was conducted to determine larval development, temporal pattern of emergence and teneral spatial distribution of the species in the Seybouse watershed, north-east Algeria. Larval growth was investigated in two populations: one at about 200 m (low-elevation population) and the second at 600 m of elevation (high-elevation population). The species showed partial bivoltine life cycle in both low- and high-elevation population. The temporal pattern of emergence of the first flight season of the year at low-elevation population was asynchronous with an emergence season lasting 46 days and half of the population emerging in 15 days. The second flight season was shorter with a most likely smaller population size. Sex ratio at emergence was slightly male biased. After ecdysis, tenerals stayed next to the water within a mean distance of 4.76 ± 4.35 m (± SD) with no significant difference between sexes. Conservation measures that should be taken into account in the elaboration of future management plans for the species are discussed.

*Key words:* damselfly, elevation, emergence, larval growth, odonata, spatial distribution

## Résumé

*Calopteryx exul* est une espèce endémique de demoiselle en danger qui subit une importante dégradation de son habitat et des extinctions locales dans toute son aire de répartition géographique. Si de récentes études concernaient sa distribution, son écologie et sa systématique à l'état larvaire, le cycle biologique de l'espèce reste inconnu. Dans cette étude, nous avons mené une recherche de terrain pour déterminer le développement des larves, le schéma temporel de leur émergence et la distribution spatiale de l'espèce au stade ténéral dans le bassin de la Seybouse, au nord-est de l'Algérie. La croissance larvaire a été étudiée chez deux populations, une à 200 m d'altitude (population de basse altitude) et l'autre à 600 m (population de haute altitude). L'espèce présentait un cycle de vie partiellement bivoltin dans les deux populations. Le schéma temporel de l'émergence de la première saison d'envol de l'année pour la population de basse altitude était asynchrone, avec une saison d'émergence durant 46 jours, la moitié de la population émergeant en 15 jours. La seconde saison d'envol était plus courte, avec une population de taille très probablement plus petite. Le *sex ratio* à l'émergence penchait légèrement en faveur des mâles. Après l'exuviation, les insectes restaient à proximité de l'eau, à une distance moyenne de 4,76 ± 4,35 m (± DS), sans différence significative entre les sexes. L'on discute de mesures de conservation qui devraient être prises en compte dans l'élaboration de futurs plans de gestion.

## Introduction

Three species of Calopterygidae are known from North Africa, of which two are of conservation concern, namely *Calopteryx virgo* Linnaeus and *C. exul* Selys (Samraoui *et al.*, 2010). The former species is largely distributed in Europe but locally critically endangered. The latter species, on the other hand, is an endangered endemic damselfly

*Correspondence:* E-mail: rassimkhelifa@gmail.com

 

that presents a patchy distribution over the Maghreb, that is Tunisia, Algeria and Morocco (Boudot, 2010). Before the discovery of the Seybouse River populations in 2007 in north-east Algeria (Khelifa *et al.*, 2011), about 32 populations had been located globally but most of these populations are thought to be currently extinct and no update of their status has been reported yet (Boudot, 2010).

Most of the information regarding the species biology, ecology and systematics has been collected from the Seybouse population, which is thought to be the largest one in the Maghreb (Khelifa, 2013; Khelifa *et al.*, 2016). At this part of its geographic distribution, preferred habitats where adults were usually recorded are relatively fast-flowing shallow water with low shading (Khelifa, 2013). In addition, mark–release–resighting study carried out on some individuals showed that both sexes are able to conduct large range movements of about 5 km to reach suitable habitats (Khelifa *et al.*, 2014). Although our knowledge on the species has increased relatively over the last decade, some aspects of the life history are still unexplored. In this study, the temporal pattern of emergence and voltinism of the species were studied based on tenerals and larvae collections. The species flight period was shown to start in early May and last until late July with a peak taking place in late May–early June. After an absence of adults in late summer, some tenerals and immature individuals were also observed in early September (Khelifa, 2013). In the current study, I try to reveal whether the autumn emerging cohort comes from partial bivoltinism or an asynchronuous emergence of the univoltine population using larval population structure and the temporal pattern of emergence.

The elevational distribution of *C. exul* is large, reaching 1240 m in Morocco (Boudot, 2008); however, in north-east Algeria it is limited between about 200 and 600 m (Khelifa *et al.*, 2011, 2016). Although the difference between the extremes of the latter region is not large, it may affect the life history pattern of the species. In fact, elevation is an environmental factor that influences life history of species of many taxa (Martin, 2001). In temperate regions, many factors change over the elevational gradient. For example, temperature, nutrient availability, atmospheric pressure and CO2 content tend to decrease whereas frequency of frost, solar radiation and annual precipitation tend to increase with elevation (Körner, 2003). Of the latter factors, temperature is a factor that changes abruptly with elevation, decreasing 5.2°C to 6.5°C every 1000 m (Colwell *et al.*, 2008). Knowing that species adapt to their local environment and adjust their life history strategies to maximize their fitness (Zammuto & Millar, 1985), it is crucial to investigate the variation in life history along geographic gradients.

One aspect of odonata emergence that has not attracted much attention despite its conservation implication is the habitat preferences of tenerals (i.e. the first day of the adult life stage). The survey of teneral spatial distribution of species of conservation concern is essential because during this period individuals are very vulnerable to physical damage due to their low flight abilities and their soft body and wings (Corbet, 1999). Therefore, to effectively conserve the species by limiting the disturbance by livestock and human, the spatial distribution of *C. exul* tenerals was investigated in this study.

I hypothesize that the life history pattern of the species is typically ‘summer species’ (Corbet, 1954) because according to previous records, the flight season is relatively long (Khelifa *et al.*, 2013). Moreover, the occurrence of bivoltinism instead of extended automnal flight season is more likely because first, there is a gap of a month (August) during which adults are not recorded and second, such voltinism has already been observed in another zygopteran within the same region (Mahdjoub *et al.*, 2015). Finally, the variation in life history along the small elevational gradient might be significant, considering the steep changes of environmental conditions.

## Material and methods

### *Study site*

The study was carried out in the Seybouse watershed, located in north-east Algeria and flows into the Mediterranean Sea (36°52′3″ N, 7°46′25″E). The larval population structure was investigated in two localities (Fig. 1): one at El Fedjoudj (low-elevation population), which is one of the lowest populations of the watershed, and one at Cheniour (high-elevation population), which is considered as the highest population of the watershed. The former site is situated upstream the Seybouse River, 5 km west from Guelma city (36°28′23.16″N, 7°22′32.73″E, 210 m a.s.l.). The watercourse is usually shallow and exceeds 10 m width. Bank vegetation is consisted mainly of *Typha*

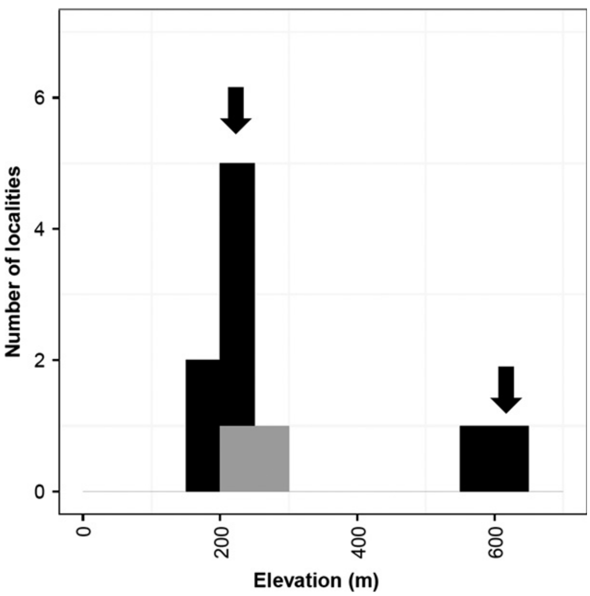


**Fig 1** Elevational distribution of known localities of *Calopteryx exul* in the Seybouse watershed (according to Khelifa *et al.*, 2016). Dark and light grey represent localities where reproduction has and has not been recorded, respectively. Black arrows refer to sampling localities

*angustifolia* L., *Cyperus longus* L., *Juncus maritimus* Lam., *Tamarix gallica* L. and *Paspalum distichum* L. The latter site is a fast-flowing stream of 3–4 m large (36°13′34.86″N, 7°19′9.74″E, 608 m a.s.l.), dominated with *Cyperus longus* and *Nerium oleander*. The coexisting odonates are *Calopteryx haemorrhoidalis*, *Platycnemis subdilatata*, *Gomphus lucasii* and *Boyeria irene*.

### *Larval population structure*

Monthly larvae collections were carried out from September 2010 to August 2013 in the two study sites using a rectangular net (40 × 25 cm) constructed from 0.5-mm mesh. A 10 m-stretch of bank vegetation, where most larvae occur (Khelifa, 2012), was sampled. Larvae smaller than F-4 were pooled together in further analyses. Body length, head width and the length of the left hind wing sheath of larvae were measured in the laboratory with a digital calliper to the nearest 0.01 mm when larvae were enough large, and with a dissecting microscope when they were small. After measurements, about 60% of larvae were returned to their original sites and the rest was conserved in 70% ethanol.

### *Temporal pattern of emergence*

This aspect was studied only at El Fedjoudj. As part of another study on the species dispersal carried out in 2011 which consisted of daily capture–mark–resighting of individual adults including tenerals along a stretch of 2 km starting on 25 April, data on teneral phenology were used to determine the temporal pattern of emergence. Exuviae were not investigated because they were difficult to detect and this would not give reliable estimations of the temporal pattern of emergence. The riparian area (up to 40 m from the water) was searched carefully for the presence of tenerals. The sex and distance from the water (to the nearest 0.5 m) were noted. The EM10, EM50 and EM90 were calculated as the time (number of days) during which 10%, 50% and 90% of the total number of tenerals were recorded over the emergence season. Occasional visits were conducted in September 2011 to check for the occurrence of a second generation by recording tenerals.

### *Statistical analyses*

R 3.1.2 (R Development Core Team, 2015) was used to perform all statistical analyses. Fisher's exact tests were carried out to test for differences in the distribution of larval instars among populations and months. Chi-squared test was conducted to determine potential significant deviation of sex ratio at emergence from the 1 : 1 ratio. Mann–Whitney *U*-test was carried out to test for significant differences in teneral distribution (the distance from the water) between males and females.

## Results

### *Larval population structure*

A total of 183 and 151 larvae were collected from low- and high-elevation population, respectively. In both populations, the larval population structure prior to winter and emergence was asynchronous (Fig. 2). There was an apparent winter diapause in both low- and high-elevation population, revealed by the nonsignificant change in the distribution of larval instars between December and February (Fisher's exact test: $P = 0.33$ and $P = 0.29$ in low- and high-elevation population, respectively). The distribution of larval instars was similar in both populations in December, when larvae entered the wintering season (Fisher's exact test: $P = 0.31$), and in April, that is

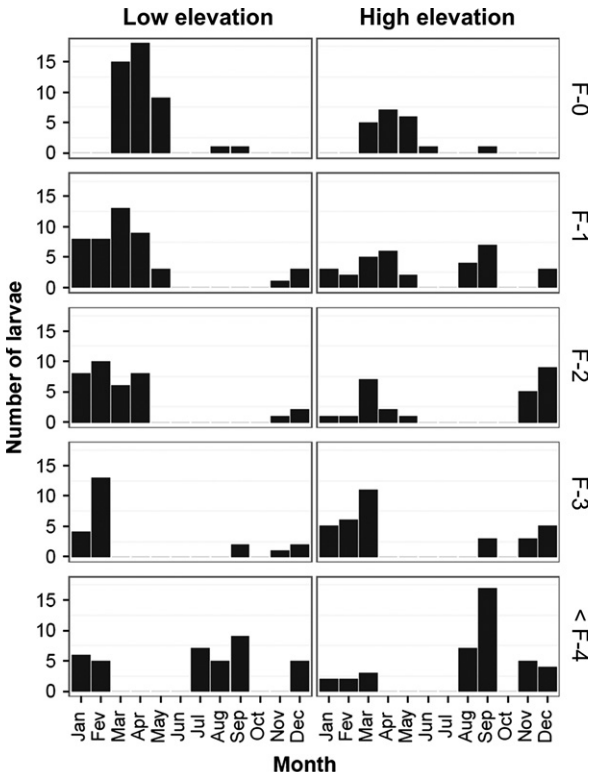


**Fig 2** Monthly larval population structure of *Calopteryx exul* in low- and high-elevation population

prior to the first emergence of the year (Fisher's exact test: $P = 0.56$) (Fig. 2). Before emergence (April), 51.4% (n = 35) of larvae were at the final instar in low-elevation population and it increased in May to 75% (n = 12) (Fig. 2). No more larvae were found in June. In high-elevation population, 46% (n = 15) of larvae were at the final instar in April and it increased to 66% (n = 9) in May and 100% (n = 1) in June. There was no larva found in July in high-elevation population (Fig. 2).

At low-elevation population, the final instar larva was recorded in August, while it was observed in September at high-elevation population (Fig. 2). However, there was a marginally significant difference among populations in September (Fisher's exact test: $P = 0.05$), during the period when the second emergence of the year starts. During this month, 91.7% (n = 12) and 71.4% (n = 28) of larvae were smaller than F-2 in low- and high-elevation population, respectively. In low-elevation population, 8.3% (n = 12) of larvae were at the final instar with no record of instars F-1 and F-2. In high-elevation population, however, only 3.6% (n = 28) of larvae were F-0 and 25% (n = 28) F-1 with no presence of F-2. However, no final instar larva was found after September in both populations (Fig. 2).

*Temporal pattern of emergence*

During the whole emergence season which lasted 46 days (from 30 April to 14 June) at low-elevation population, 551 tenerals were recorded including 282 males and 269 females. Figure 3 presents the cumulative per cent of the species emergence. EM10, EM50 and EM90 were recorded after 7.9, 15.2 and 29 days of emergence, respectively, when both sexes were included. When we consider sexes separately, EM10, EM50 and EM90 were more or less one day earlier in females (7.6, 14.5 and 28.3 days, respectively) than males (8.2, 15.6 and 29 days, respectively). Sex ratio was not significantly male biased (chi-squared test: $\chi^2 = 0.30$; $P = 0.58$), showing an overall male percentage of 51.1%. In 2011, 21 tenerals (nine males and 12 females) were recorded from 2nd to the 14th September at low-elevation population, whereas nine tenerals were observed on 27th September at high-elevation population.

*Teneral spatial distribution*

At low-elevation population, a total of 509 tenerals (92.3%) were recorded perched on the bank vegetation at distances lower than 1 m (Fig. 4). Only 42 (7.6%) individuals were noted out of the watercourse at distances ranging from 1 to 25 m with a mean of 4.76 ± 4.35 m. There was only one case where the distance was 25 m and it occurred during river flooding. There was no significant difference in the distance from the water between males and females ($U = 180$, $P = 0.32$; only distances higher than 1 m were considered).

## Discussion

This study revealed that the life history pattern of the Maghreb endemic *C. exul* at two elevations was partially bivoltine, showing a slight interpopulation difference. The investigation on both adults and larvae showed that the species was of summer species type.

Most of the life history patterns known of Calopterygidae from the western Palearctic were reported as univoltine or semivoltine (Corbet, Suhling & Soendgerath, 2006). This is not surprising as there has been no study that investigated

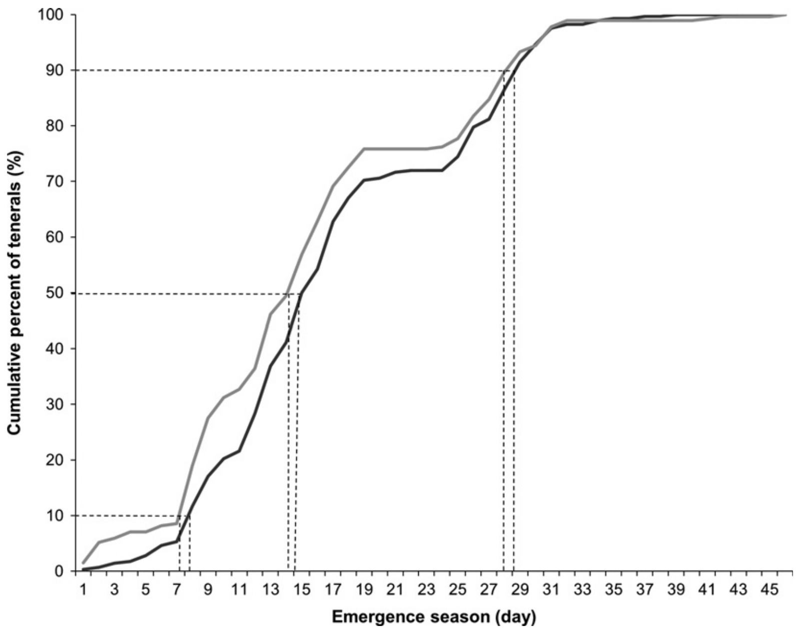


**Fig 3** Cumulative percentage of tenerals of *Calopteryx exul* during emergence season. Dark and grey lines refer to females and males, respectively. Dashed lines indicate EM10, EM50 and EM90 of both sexes

**Fig 4** A teneral male of *Calopteryx exul* perched at 1 m from the water. This individual was kept for a few hours in a cage and then marked

North African populations, where the climate is among the warmest in the temperate zone. In the present study, the analysis of the larval structure and teneral occurrence revealed that voltinism of *C. exul* is a mixture of two different life history strategies in which the population is subdivided into a slow-growing univoline cohort and a fast-growing bivoltine cohort. The fact that no larva was recorded in June and July at low-elevation population and in July at high-elevation population determines that the entire population emerged. Afterwards, the final instar larva was recorded in late August and September at low- and high-elevation population, respectively, which gives an estimation of about three to 4 months for the entire larval development if egg hatching occurs in early June. Experimental studies are needed to estimate the duration of larval development. This fast development is probably due to the fact that water temperature during July and August is optimal for larval growth and development (review in Suhling, Suhling & Richter, 2015). Similar life history pattern was also observed in *Coenagrion mercuriale* in the same watershed (Mahdjoub *et al.*, 2015), where the first generation starts to emerge in mid-spring and the second in late summer. Furthermore, according to the proportion of the three last larval instars with respect to the entire population, it is apparent that the size of the population emerging in September (second generation) is smaller than the one emerging in the spring of the following year (first generation). The occurrence of bivoltinism is probably plastic, depending primarily on environmental conditions of the year, and given the low proportion of the bivoltine population with respect to the entire population, it is reasonable to suggest that in years with low temperature or low food availability the species might be univoltine. In addition, it is likely that at higher elevations like the population in Morocco occurring at 1260 m a.s.l. (Boudot, 2008) where temperature is much colder, the bivoltinism is not probable.

Contrary to my expectation, the elevational range of the current population was not large enough to create prominent life history differences among populations. In general, there was no significant difference in the structure of larval population between low- and high-elevation population, except in the summer. It appeared like during the summer, larvae developed faster at low-elevation population and that they reached the final instar before those at high-elevation population, probably due to the differences in temperature (Lutz, 1968; Suhling, Suhling & Richter, 2015). However, prey availability differences among populations cannot be set aside because the latter has been shown to influence development rate (Pickup & Thompson, 1990) and thus may create a temporal lag between the two populations.

Similar to my expectation, the emergence season of the first generation at low-elevation population was quite asynchronous (EM50 = 15.2 days), which is typical of summer species, but it was more synchronized than its congeneric *Calopteryx haemorrhoidalis* in the Sierra Morena in which EM50 was three times greater (EM50 = 44.5 days) (Ferreras-Romero, Atienzar & Corbet, 2000). Even though the emergence of high-elevation population was not surveyed, I expect it to be as asynchronous as the elevation given the population structure of larvae before the emergence season. The second generation which has not been surveyed regularly, however, should not be asynchronous because first, larval instar distribution is not segregated to more than the two last instars, and second the emerging population is small. The slightly male biased sex ratio of *C. exul* is typical in Zygopterans (Corbet & Hoess, 1998), and it suggests different mortality rate among sexes during the aquatic stages. Such difference in mortality between males and females could be explained by differences in behavioural activities or habitat use.

The spatial distribution of tenerals may give some hints about the maiden flight, that is the distance between the site where the ecdysis takes place and the point of arrival of the first flight of the adult life stage. The species showed relatively short maiden flights, as was already recorded in three Calopterygidae by Heymer (1972). The short distance travelled is likely to be due to the low frequency of wing beats as was reported by Rüppell & Hilfert-Rüppell (2010). There was only one case when a teneral was observed at 25 m from the water, but it was probably due to the flooding that took place on that day. Once mature, both males and females spend probably all their lifespan, including reproductive behaviour, foraging and roosting next to the water (R. Khelifa, unpublished data).

This study showed that *C. exul* exhibits partially bivoltine life cycle with a large population during the first generation and a very small population during the second generation. Knowing that the species occur only in North Africa, I expect that other populations existing at around the same elevation may have similar life history. Future conservation plans should take into account that the species reproduces twice a year, that is in late spring to mid-summer and in late summer to early autumn. Therefore, disturbance due to human or livestock should be restricted by setting up physical barriers all year long so that bank vegetation will not be degraded and tenerals which show marked preferences to stay next to the water will not be damaged. The conservationists of threatened species of odonates worldwide should consider teneral habitats and define a buffer zone in their management plans.

## Acknowledgements

I am indebted to many students from Guelma University who helped me during the field work, particularly Rabah Zebsa, Hichem Amari, Mohamed Khalil Mellal, Amin Kahalerras and Soufyane Bensouilah. I am also thankful to Nasr Eddine Sakrane, Abdeldjalil Youcefi and Billel Bouregbi for transporting us to the field site and back home. Thanks also to Hayat Mahdjoub for data entering and organization.

## References

Boudot, J.P. (2008) *Selysiothemis nigra* (Vander Linden, 1825), nouveau pour le Maroc, et autres observations sur les Odonates du maghreb nord-occidental (Odonata: Anisoptera: Libellulidae). *Martinia* **24**, 3–29.

Boudot, J.P. (2010) *Calopteryx exul*. The IUCN Red List of Threatened Species.

Colwell, R.K., Brehm, G., Cardelús, C.L., Gilman, A.C. & Longino, J.T. (2008) Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science* **322**, 258–261.

Corbet, P.S. (1954) Seasonal regulation in British dragonflies. *Nature* **174**, 655.

Corbet, P.S. (1999) *Dragonflies: Behaviour and Ecology of Odonata*. Harley Books, Colchester, UK.

Corbet, P.S. & Hoess, R. (1998) Sex ratio of Odonata at emergence. *Int. J. Odonatol.* **1**, 99–118.

Corbet, P.S., Suhling, F. & Soendgerath, D. (2006) Voltinism of Odonata: a review. *Int. J. Odonatol.* **9**, 1–44.

Ferreras-Romero, M., Atienzar, M.D. & Corbet, P.S. (2000) Voltinism of *Calopteryx haemorrhoidalis*, (Vander linden) in the sierra Morena Mountains, Southern Spain (Zygoptera: Calopetrygidae): a preliminary study. *Int. J. Odonatol.* **3**, 125–130.

Heymer, A. (1972) Comportements social et territorial des Calopterygidae (Odon. Zygoptera). *Soc. Entomol. France Ann. (ns)* **8**, 3–53.

Khelifa, R. (2012) Description of the final instar larva of *Calopteryx exul* Sélys, 1853 (Zygoptera: Calopterygidae). *Int. J. Odonatol.* **15**, 107–114.

Khelifa, R. (2013) Flight period, apparent sex ratio and habitat preferences of the Maghribian endemic *Calopteryx exul* Selys, 1853 (Odonata: Zygoptera). *Revue d'écologie* **68**, 37–45.

Khelifa, R., Youcefi, A., Kahlerras, A., AL Farhan, A., AL-Rasheid, K. A. & Samraoui, B. (2011) L'odonatofaune (Insecta: Odonata) du bassin de la Seybouse en Algérie: intérêt pour la biodiversité du Maghreb. *Revue d'écologie* **66**, 55–66.

Khelifa, R., Mahdjoub, H., Zebsa, R., Kahalerras, A., Guebailia, A., Amari, H. & Houhamdi, M. (2013) Aspects of reproductive biology and behaviour of the regional critically endangered *Urothemis edwardsii* (Odonata: Libellulidae) on Lake Bleu (Algeria). *Zool. Ecol* **23**, 282–285.

Khelifa, R., Zebsa, R., Sakrane, N.E., Youcefi, A., Bensouilah, S. & Amari, H. (2014) Long-range movements of an endangered endemic damselfly *Calopteryx exul* Selys, 1853 (Calopterygidae: Odonata). *Afr. J. Ecol.* **52**, 375–377.

Khelifa, R., Zebsa, R., Amari, H., Mellal, M. K., Mahdjoub, H. & Kahalerras, A. (2016) A hotspot for threatened Mediterranean odonates in the Seybouse River (Northeast Algeria): are IUCN population sizes drastically underestimated? *Int. J. Odonatol*. In press. doi: 10.1080/13887890.2015.1133331.

Körner, C. (2003) *Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems; with 47 Tables*. Springer Verlag, Berlin, Germany.

Lutz, P. E. (1968) Effects of temperature and photoperiod on larval development in *Lestes eurinus* (Odonata: Lestidae). *Ecology*. **49**, 637–644.

Mahdjoub, H., Khelifa, R., Zebsa, R., Bouslama, Z. & Houhamdi, M. (2015) Bivoltinism in *Coenagrion mercuriale* (Zygoptera: Odonata) in the southern margin of its distribution range: emergence pattern and larval growth. *Afr. Entomol.* **23**, 59–67.

Martin, K. (2001) Wildlife communities in alpine and subalpine habitats. In: *Wildlife-Habitat Relationships in Oregon and Washington* (Eds. D.H. Johnson and T.A. O'Neil). Oregon State University Press, Corvallis, OR, U.S.A.

Pickup, J. & Thompson, D.J. (1990) The effects of temperature and prey density on the development rates and growth of damselfly larvae (Odonata: Zygoptera). *Ecol. Entomol.* **15**, 187–200.

R Development Core Team. (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rüppell, G. & Hilfert-Rüppell, D. (2010) Kinematic analysis of maiden flight of Odonata. *Int. J. Odonatol.* **13**, 181–192.

Samraoui, B., Boudot, J. P., Ferreira, S., Riservato, E., Jovic, M., Kalkman, V. J. & Schneider, W. (2010) The Status and distribution of dragonflies. In: *The Status and Distribution of Freshwater Biodiversity in Northern Africa* (Eds. N. Garcia, A. Cuttelod and D. Abdul Malak). 51–70. IUCN, Gland, Switzerland.

Suhling, F., Suhling, I. & Richter, O. (2015) Temperature response of growth of larval dragonflies – an overview. *Int. J. Odonatol.* **18**, 15–30.

Zammuto, R. M. & Millar, J. S. (1985) Environmental predictability, variability, and *Spermophilus columbianus* life history over an elevational gradient. *Ecology*. **66**, 1784–1794.

(*Manuscript accepted 29 April 2016*)

doi: 10.1111/aje.12332