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ORIGINAL PAPER

On the restoration of the last relict population of a dragonfly *Urothemis edwardsii* Selys (Libellulidae: Odonata) in the Mediterranean

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Abstract The restoration of endangered relict populations is challenging in conservation biology because they require specific environmental conditions within an inhospitable regional climate. *Urothemis edwardsii* Selys is the most endangered dragonfly in the Mediterranean with only one known relict small population (Lac Bleu) left in Northeast Algeria. With the absence of successful (re-) colonization over the last two decades, the restoration of the species became a top priority. To improve the status of the species in Northeast Algeria, we carried out a reintroduction and translocation scheme during 2011–2015 and assessed the changes in distribution and population size. Our restoration plan led to the emergence of three populations of which one was restored (Lac Noir), one resulted from successful translocation (Lac Tonga Northeast), and one established after successful colonization (Lac Tonga Southwest). In three localities (Lac Noir, Lac Tonga Northeast, and Lac Tonga Southwest), signs of population growth were observed, whereas no significant trend in the source population (Lac Bleu) was detected. A new population (El Graeate) was also recorded in 2015, but its origin is uncertain. Capture-mark-recapture on adults conducted in 2015 in two sites (Lac Bleu and Lac Noir) showed low

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recapture rates and no sign of dispersal between the two sites. Dispersal capacity of the species and conservation implications of adult distribution are discussed. This study highlights the importance of using biological indicators in selecting host habitats for the restoration of critically threatened populations.

Keywords Conservation · Reintroduction · Translocation · Colonization · Odonata · Dragonfly · Wetlands · North Africa

Introduction

Restoration plans including translocation and re-introduction of threatened species have been widely applied for the conservation of animals in different biotopes (Seddon et al. 2007, 2012). Even though most attention has been devoted to vertebrates such as mammals (Kleiman 1989), birds (Sutherland et al. 2010), and amphibians (Trenham and Marsh 2002), some studies have also focused on the conservation of invertebrates like lepidopterans (Andersen et al. 2014; Fred and Brommer 2015) and odonates (Hannon and Hafernik 2007; Thompson et al. 2015). The procedure which consists of moving individuals from one site to another is simple, but the success of the process in the long term has always been challenging (Fischer and Lindenmayer 2000; Wimberger et al. 2009). Therefore, a good understanding of species habitat requirements and ecological needs are prerequisites for the success of species restoration.

Urothemis edwardsii (Selys 1849) is listed as critically endangered in the Mediterranean IUCN red list (Riservato et al. 2009), and is certainly the most threatened dragonfly regionally. This afrotropical species is common in central and southern Africa, but occurs in North Africa as relict in the coastal areas where the climate is similar to the tropics. Many populations have become extinct in Algeria, Tunisia, Israel, Palestine and Jordan during the last two decades (Boudot et al. 2013), probably due to anthropogenic pressure. In Algeria, there are two known localities of *U. edwardsii*, of which one (Lac Noir) was recorded extinct in 1990 (Samraoui et al. 1993), and one (Lac Bleu) had last been observed in 2006 and assumed on the verge of extinction or possibly extinct by Riservato et al. (2009). Later records have confirmed that the species still exists in Lac Bleu (Khelifa et al. 2013a, b), and this represents the only known population living today in the entire Mediterranean basin.

There are two major concerns about the survival of the last living population of *Urothemis edwardsii* of the Mediterranean. First, the inability of the species to colonize new areas during the last two decades (Riservato et al. 2009) suggests that the species has low dispersal capacities. This is due to either high philopatry to emergence sites (i.e. individuals reproduce at the same site where they emerge), low movement abilities (i.e. unable to move from one site to another) or low colonization success (i.e. able to move and lay eggs in other sites but unable to survive up to the adult stage). The second concern is that the last living population occurs in a 2 ha-waterbody which is probably highly vulnerable to anthropogenic effects and has a small carrying capacity for larvae and adults. These characteristics put the species under high extinction risk, and human intervention seems a necessity for its regional persistence.

Recently, IUCN experts concluded that “A species action plan for the CR and relict species, *Urothemis edwardsii*, is urgently needed” (Riservato et al. 2009). There are two options to restore the species. The first one is to reintroduce it in areas where it used to exist. In Northeast Algeria, Lac Noir is the only site that used to support a population until the early 1990s when dryness and fire degraded the bog and the entire landscape (De Belair and Samraoui 1994). The second option is to translocate the species to new sites where the species has never been recorded.

Here we present an updated distribution of *U. edwardsii* in Northeastern Algeria, and report the discovery of two new populations. We applied a reintroduction-translocation scheme based on larvae and eggs between 2011 and 2015 to restore and expand the range of the species. We conducted a reintroduction of the species in Lac Noir. After selecting the most appropriate site for translocation (see Material and methods), the latter was conducted in Lac Tonga. Finally, to determine the degree of philopatry and movement abilities of the species capture-mark-recapture (CMR) was carried out during one season at two different sites (Lac Bleu and Lac Noir).

Materials and methods

Study sites and sampling

The study was conducted in Northeast Algeria (Fig. 1). This area has a Mediterranean climate, characterized by a wet season from October to March and a dry season from April to August/September with an annual rainfall of 710–910 mm. A total of 15 sites were surveyed in 2011–2015 (Table 1) and visited at least three times during the flight season of the species (June–August) from 09:00 to 15:00. Visits were conducted only when weather was good. We recorded the number of adults and the occurrence of reproduction (tandems, copulation and oviposition) and exuviae. The number of adults were estimated by walking along a 100 m transect near the shore. In sites where adults were found, substantial efforts were conducted to find exuviae in the water body.

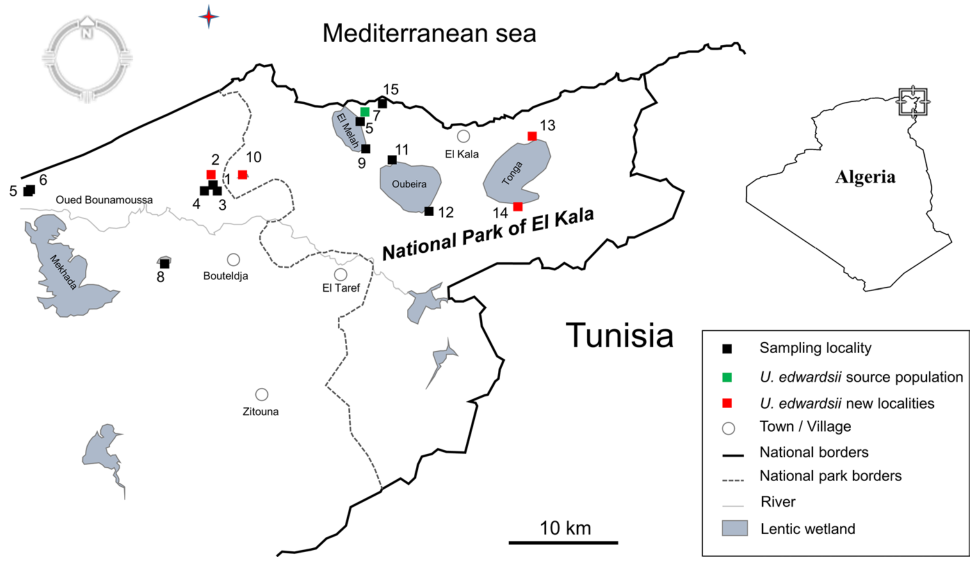

Fig. 1 Study site showing populations of *Urothemis edwardsii* in Northeast Algeria. Geographic coordinates and the name of sites are presented in Table 1

Table 1 Geographic coordinates and area of the study sites

Code	Site	Geographic coordinates	Area (ha)
1	El Graete 1	36.848581°, 8.176275°	10
2	El Graete 2	36.853941°, 8.175466°	35
3	El Graete 3	36.841564°, 8.169550°	1.3
4	El Graete 4	36.843478°, 8.178520°	3.2
5	G. Dakhla	36.844801°, 7.986809°	6.2
6	G. Estah	36.843605°, 7.983178°	3.6
7	Lac Bleu	36.909292°, 8.338283°	2
8	Lac des oiseaux	36.781451°, 8.125407°	70
9	Lac Mellah	36.880944°, 8.342711°	870
10	Lac Noir	36.854725°, 8.206829°	2.2
11	Lac Oubeira 1	36.865286°, 8.364081°	2198
12	Lac Oubeira 2	36.824590°, 8.403702°	2198
13	Lac Tonga NE	36.881639°, 8.525145°	2700
14	Lac Tonga SW	36.826686°, 8.486934°	2700
15	Ruppia	36.916939°, 8.344031°	0.21

Choice of host habitats

Due to the small population size of Lac Bleu, we planned our restoration only for two sites (Lac Noir and Lac Tonga NE).

Lac Noir

Although Lac Noir was subject to complete dryness and fire of both the dry bog and the surrounding vegetation in the early 1990s (De Belair and Samraoui 1994), there is evidence that the site has recovered during the past 25 years. One sign of recovery was the rediscovery of a local reproductive population of an endangered afrotropical relict dragonfly *Acisoma inflatum* (Ex. *A. panorpoides ascalaphoides*, Mens et al. 2016; Khelifa unpublished data) in 2010, after being reported extinct 20 years before (De Belair and Samraoui 1994). Further visits in 2015 found that the species was quite abundant. This recolonization of Lac Noir was probably the result of a successful dispersal from other nearby populations, as *A. inflatum* is quite abundant in the region. Since *U. edwardsii* coexisted with *A. inflatum* and went extinct at the same time, we used the latter species as a bioindicator of habitat quality improvement and hypothesized that there was a chance that *U. edwardsii* would survive in Lac Noir as well.

Lac Tonga

The study species had never been observed in Lac Tonga before our translocation. The choice of this site was based on four criteria, namely local odonatofauna, local plant community, size and protection status. The Northeastern part of the lake (Lac Tonga NE) as a biotope represents a larger version of Lac Bleu, presenting a similar odonatofauna and plant community. The analyses of exuviae, which represent the most accurate indicator of successful reproduction in a site (Raebel et al. 2012), collected between 2010 and 2011 showed that all zygopterans (7 species) and 92.3 % of anisopterans (12 species) that were found in Lac Tonga also lived in Lac Bleu (Table S1), that is, to Lac Tonga had a similar odonate composition as Lac Bleu, except for the absence of *U. edwardsii*. Regarding the plant community, all species that are used by *U. edwardsii* larvae for emergence (*Nymphaea alba, Cladium mariscus, Phragmites australis* and *Iris pseudacorus*) were also found in Lac Tonga NE. Furthermore, with an area of 2,700 ha, this lake has potentially greater carrying capacity than that of the source population of Lac Bleu (2 ha) and may support a much larger population of *U. edwardsii*. Finally, this site is an integral natural reserve protected since 1983 (Loukkas 2006) which might help in the persistence of the species in the future.

Reintroduction and translocation scheme

To improve the status of the species, both reintroduction and translocation were conducted. *Urothemis edwardsii* was reintroduced to Lac Noir and translocated to Lac Tonga. Both eggs and final instar larvae, collected from Lac Bleu, were used in these experiments which took place in 2011–2013 and 2015. Table 2 gives the number of larvae and the estimated number of eggs translocated to each site. The maximum number of larvae collected per year from Lac Bleu to conduct the restoration measures was 54 in 2013, which we think represents about 10 % of the total population size estimated to be around 250 breeding pairs by Riservato et al. (2009). We transferred only final instar larvae to increase the survival probability until emergence, as was conducted for an endangered damselfly (Khelifa 2012). The transfer of larvae was carried out in early May, prior to emergence. Eggs were obtained by capturing females in copula and immersing the tip of their abdomen in a vial containing water. The estimation of clutch size was conducted by combining information on egg deposition rate and oviposition time (see Khelifa et al. 2012). Since we marked females after each egg collection, we knew that every clutch came from a different female. This was important not only to avoid catching females more than once and limiting the potential survival costs due to handling (Cordero et al. 2002), but also to increase genetic diversity of the host population (Frankel and Soulé 1981). Egg clutches were translocated in July and August within 2 h of their collection. Both eggs and larvae were translocated to specific areas where water depth was about 2 m and waterlilies (*Nymphaea alba*) were abundant, which corresponds to the preferred emergence site of the species (Khelifa et al. 2013b). To avoid cannibalism, larvae and eggs were spread across different patches. According to our observations, the species is univoltine with a flight season that starts from mid-May to late August. So we expected that (1) the final instar larvae emerge in the same year of translocation, (2) translocated eggs also hatch in the same year (Khelifa et al. 2013a), and (3) newly hatched larvae emerge in the following year.

Table 2 Number of eggs and larvae transferred to Lac Noir and Lac Tonga

Restoration measure	Site	Year	Number of eggs estimated	Number of larvae	Females (%)
Reintroduction	Lac Noir	2012	7210 (16)	23	52.1
		2013	6835 (13)	27	55.5
		2015	4516 (11)	23	56.5
Translocation	Lac Tonga	2011	5823 (12)	46	54.3
		2012	8530 (15)	23	43.4
		2013	3521 (7)	27	44.4
		2015	0	23	52.1

Numbers between brackets are the number of clutches

Dispersal and spatial distribution

In 2015, we conducted CMR twice a week from 30 July to 26 August in Lac Bleu and Lac Noir. Two surveyors spent 2 h in each site between 10:00 and 15:00, when species activity is at its peak. In each site, imagos were captured with hand nets and marked with a permanent marker on one of the hind wings with an individual number. During the same year, the spatial distribution of mature adults was surveyed in 2015 in Lac Bleu and Lac Noir by estimating the distance from the water shore of perching sites. In each site, a large area around the wetland was checked for the presence of adults (up to 1.5 km from the water).

Statistical analyses

Statistical analyses were performed with the software R 3.2.1 (R Development Core Team 2016). Simple linear regression was used to assess the temporal pattern of the estimated number of individuals in four populations. A two-sample Kolmogorov–Smirnov test (K–S) was used to test whether the distribution of distances from the water of *U. edwardsii* adults was different between Lac Bleu and Lac Noir.

Results

Geographic distribution

The number of sites where *U. edwardsii* occurs increased from one in 2011 to five in 2015 (Table 3). We recorded four new reproductive populations, namely Lac Noir, Lac Tonga NE, Lac Tonga SW and El Graeate. Of the four new sites, Lac Tonga NE and Lac Noir populations were restored after reintroduction in 2011 and 2012, respectively, and Lac Tonga SW emerged in August 2012 without our intervention. The later site is 7 km away from Lac Tonga NE. In these three sites, indicators of successful reproduction including reproductive pairs, copulation, oviposition or exuviae were recorded (Table 3). The new population of El Graeate, which does not belong to our restoration scheme, was discovered in July 2015 where more than ten reproductive pairs and 23 exuviae were recorded. This new site is 2.5 km away from the nearest source population (Lac Noir). Regarding the distance between all populations of *U.*

Table 3 Records of *Urothemis edwardsii* in each sampling visit to five localities between 2011 and 2015

Year	2011			2012			2013			2015		
Sampling visit	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug	Jun	Jul	Aug
Lac Bleu	ATE	ARE	A	AT	ATR	ARE	AE	ARTE	ARE	ARTE	ARE	ARE
Lac Noir	0	0	0	0	0	0	ATE	ART	A	AT	ART	A
Lac Tonga NE	0	0	0	A	A	ART	A	ART	AR	AT	ART	AR
Lac Tonga SW	0	0	0	0	AT	A	A	AT	A	ART	AR	A
Graeate	–	–	–	–	–	–	–	–	–		ARET	ARE
No.localities	1			3			4			5		

– means that the site was not sampled, 0 refers to the absence of the species, *A* adult, *T* teneral, *R* reproduction (copulation and/or oviposition), *E* exuvia

edwardsii, the mean of all pairwise distances between the five sites was 18.25 ± 9.57 km (N = 10), with a minimum of 2.5 km between Lac Noir and El Graeate, and a maximum of 31.5 km between Lac Tonga NE and El Grarate (Fig. 2).

Population trend

The temporal patterns of the estimated number of individuals from 2011 to 2015 in four populations are shown in Fig. 3. The four populations showed different temporal patterns (Table 4), with no significant change in the source population (Lac Bleu) (linear model: r = −0.21, P = 0.6), and a positive increase in the three other populations. The magnitude of the increase was higher in the two restored populations (Lac Noir and Lac Tonga NE) in which regular transfers of larvae and eggs (augmentation) were conducted.

Fig. 2 Pairwise distance between the five sites of *U. edwardsii*

The estimated number of individuals increased by a factor of 3.2 (linear model: SE = 0.46, P < 0.0001) in Lac Tonga NE and 2.56 in Lac Noir (linear model: SE = 0.53, P < 0.0001). In the newly established population (Lac Tong SW), the estimated number of individuals showed a slower increase with a slope of 1.46 (linear model: SE = 0.52, P = 0.01).

Dispersal and spatial distribution

A total of 102 (94 males and 8 females) and 49 individuals (48 males and 1 female) were marked during ten sampling occasions in Lac Bleu and Lac Noir, respectively. Of the 102 individuals marked in Lac Bleu, 8 (7.8 %) were recaptured within the same site, with seven (7.4 %) males and one female (12.5 %) resighted. Only one male (0.01 %) was resighted on two different occasions. Of the 49 individuals marked in Lac Noir, 6 (12.2 %) were resighted in the same site and all were males (12.7 %). In this site, no male was recorded more than once. No movement from one site to another was recorded.

Males of *U. edwardsii* usually perch on supports away from the water (Fig. 4). The distance of adults from the water shore had a mean of 39.63 ± 137.76 m (1–1,240 m, N = 105) in Lac Bleu and 17.38 ± 18.56 m (1–89 m, N = 49) in Lac Noir. The distribution of distances was significantly different (K–S test: D = 0.36, P = 0.0002), showing a more right-skewed distribution in Lac Bleu (Fig. 5).

Discussion

Our study provides an update to the status of *U. edwardsii* in the last standing relict population of the Mediterranean and reports preliminary results of a restoration scheme. Four new reproductive populations were recorded, of which one was restored (Lac Noir), one resulted from successful translocation (Lac Tonga NE), one established probably after successful colonization (Tonga SW), and one with an uncertain origin (El Graeate). There is evidence of population

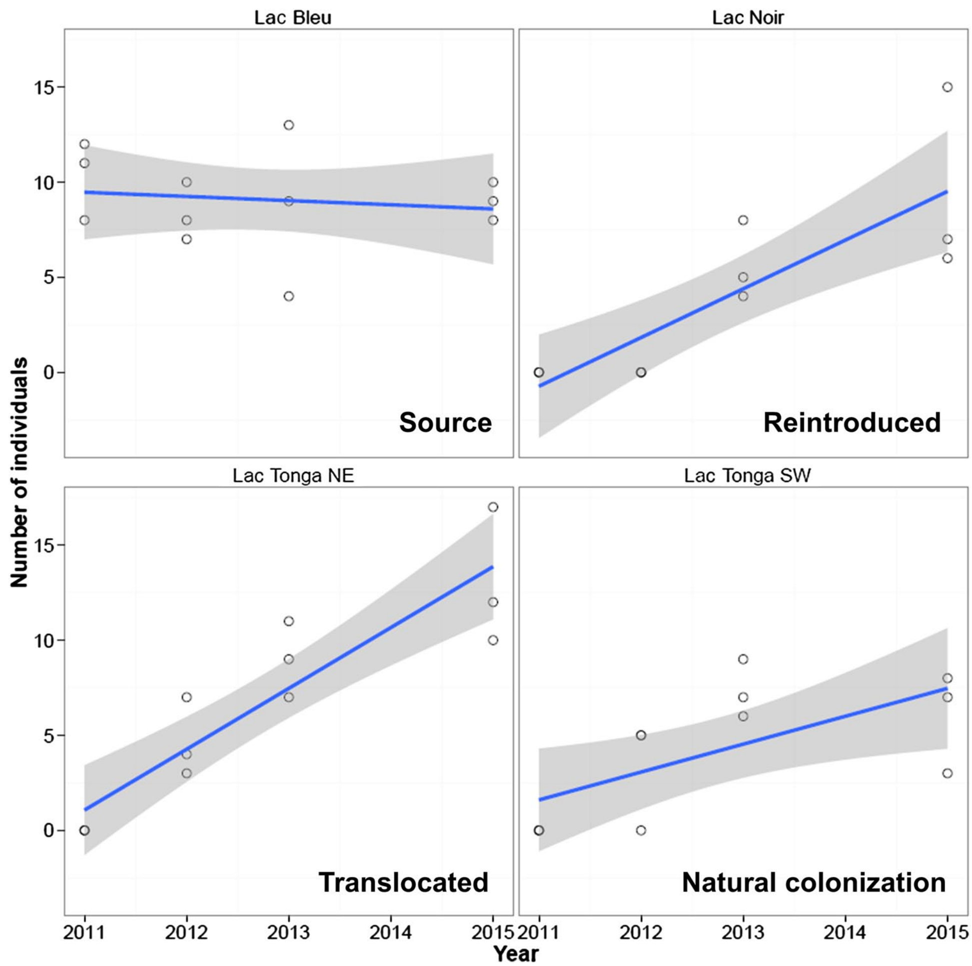

Fig. 3 Population trend over time in four sites of *U. edwardsii*. Regression lines are in *blue* and the ribbons indicate the 95 % confidence intervals. (Color figure online)

Table 4 Summary results of the linear model of population trend of *U. edwardsii* in four populations

	Estimate	Std. error	t value	P
Intercept	449.9714	1010.5564	0.445	0.658
Year	−0.2190	0.5021	−0.436	0.664
Site Lac Noir	−5602.6952	1429.1426	−3.920	0.0003
Site Lac Tonga NE	−6884.1048	1429.1426	−4.817	<0.0001
Site Lac Tonga SW	−3397.8381	1429.1426	−2.378	0.02
Year:Site Lac Noir	2.7810	0.7100	3.917	0.0003
Year:Site Lac Tonga NE	3.4190	0.7100	4.815	<0.0001
Year:Site Lac Tonga SW	1.6857	0.7100	2.374	0.022

The population in the intercept is Lac Bleu

growth in three of the new populations, and stability of the source population between 2011 and 2015. CMR showed low recapture rates and no dispersal from one site to another (13 km apart). Finally, our analysis of adult distribution suggests that the species occupies a large area of terrestrial habitat around the wetland.

Our successful reintroduction and translocation confirm that the potential distribution range of the species was larger, and that the inability to successfully colonize new areas was possibly due to environmental factors that limited the dispersal of *U. edwardsii*, as was found in other dragonflies (McCauley 2006). The new population in Lac Tonga SW was probably the result of successful colonization because it was not observed in 2011 and was first detected in 2012, a year after the translocation of the species to Lac Tonga NE. Furthermore, the origin of the new population of El Graeate remains uncertain since we do not know whether it has simply been overlooked or was recently established. When we refer to the literature, it becomes clear that the site was not sampled by previous investigations of the region (Samraoui and Menaï 1999; Samraoui and Corbet 2000). However, it is hard to believe that this population has been overlooked for 25 years given the fact that the species has a conspicuous behavior, easily identifiable and occupies a large terrestrial habitat around emergence sites. Therefore, we should not ignore the possibility that individuals from our successfully reintroduced population to Lac Noir, which is 2.7 km away from El Graeate were able to disperse and colonize the pond.

Fig. 4 Mature male of *U. edwardsii* perched at about 200 m from the water in Lac Noir (July 2013)

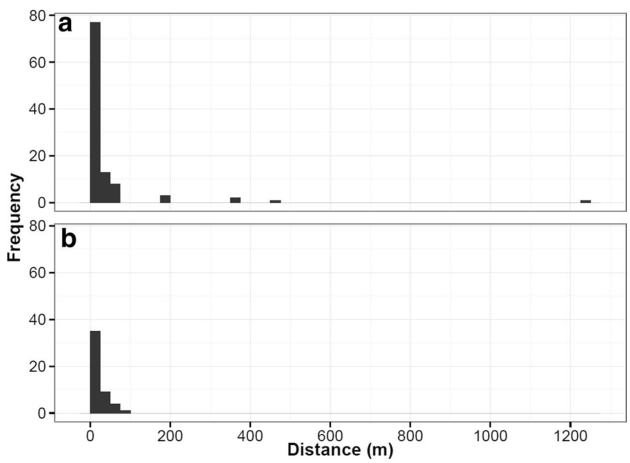

Fig. 5 Frequency distribution of distance from the water of adult *Urothemis edwardsii* in Lac Bleu (**a**) and Lac Noir (**b**). Bins are set to 25 m

The dispersal ability of the species might have been underestimated in the past. Two things make us suggest that *U. edwardsii* may travel long distances. First, the very low recapture rate of the species was probably not only the result of mortality but also of dispersal. Second, the successful colonization of Lac Tonga SW revealed a dispersal capacity of 7 km. The reason why *U. edwardsii* could not disperse from Lac Bleu to Lac Noir (13 km) or Lac Tonga (17 km) without our intervention is probably not just due to the distance involved but also to the physical barriers of the local landscape as was documented for other organisms (Dover and Settele 2009). For instance, to fly from Lac Bleu to Lac Tonga the species has to fly through large areas of bushes (8 km long), urbanized areas (3–4 km long), an oak forest (3–4 km long) and landforms that are 200 m higher than the original site. These are barriers known to limit the movement of odonates (Corbet 1999; Purse et al. 2003). However, other biotic factors could also influence its dispersal like population density, predation risks and body condition (Clobert et al. 2001; Baines et al. 2014). An extensive CMR study is needed to understand the movement capacities and factors that might influence the dispersal of *U. edwardsii*.

Our restoration program which consisted of combining both final instar larvae (<10 % of the source population each year) and eggs was successful. The use of the final instar larvae was crucial because the mortality probability until emergence is low, compared to eggs (Corbet 1999). The transfer of eggs was also important because it probably contributed to the increase in population size and reduced the overharvesting of larvae of the source population which represent the initial population size of the adult stage. The success of the restoration procedure was reflected by two positive outcomes. First, our restoration measures increased the population size of the host populations quite remarkably, similar to what was reported for the translocated field cricket (*Gryllus campestris* L. 1758) in Northern Germany (Hochkirch et al. 2007). It seems that *U. edwardsii* found an empty ecological niche in the host habitats and occupied it successfully. Second, our method did not show any sign of overharvesting of the source population since no significant negative trend of the source population size was observed. This reveals that our restoration program was based on sustainable harvest levels that should be taken into account in the conservation plans of other threatened odonates and macroinvertebrates.

We documented the distribution of adults in terrestrial habitats by estimating the distance from the nearest emergence sites. We found that *U. edwardsii* males could wander more than 1 km away from the water. Such information is very valuable from a conservation perspective because it allows conservationists to consider relatively large buffer zones surrounding the water body in their management plans (Sutherland et al. 2010). The difference in the distance from the water between Lac Bleu and Lac Noir was probably the result of population size. Our CMR showed that the population of *U. edwardsii* in Lac Bleu is currently much larger than that of the restored population of Lac Noir. The longer distances recorded in Lac Bleu could be the result of intraspecific competition for space among males (McCauley 2010), as the species is known to be territorial (Khelifa et al. 2013a).

The future of the species seems promising since there is evidence of range expansion and population growth. The good news is that both El Graeate and Lac Tonga are 17.5 and 1,000 times larger than Lac Bleu, respectively, and probably have larger carrying capacities. We expect that the new populations will continue to grow and even expand the range. However, there is an urgent need to conduct regular estimations of population size in all localities to understand the population dynamics of the species, and track the evolution of spatial distribution over time to assess range dynamics.

Possible threats

The conservation measures have to be strengthened more than ever to ensure the persistence of the new and old populations. Some major threats including water pumping and livestock grazing of emergence sites were recorded during our survey (Fig. S1). Newly introduced populations are very vulnerable during the first years (Hannon and Hafernik 2007), and probably require several years to fully establish a viable population. Another obvious threat to our populations is the potential low genetic diversity and the possibility of future inbreeding depression (Westemeier et al. 1998; Vilà et al. 2003; Hedrick and Fredrickson 2010). In fact, inbreeding depression is known to increase the extinction risk of small populations (Brooks et al. 2002). This issue is unavoidable since only one small population was left in the Mediterranean. Although this major problem has been addressed widely (Howard 1993; Hedrick 1995), its direct effect on population viability remains questionable (Caro and Laurenson 1994; Caughley and Gunn 1996).

Conclusion

Our study represents a good conservation exercise because we started from the worst case scenario in conservation biology where only one small population was left and very little information on the species was available. We highlighted the importance of the use of bioindicators and knowledge of the natural history of species in the application of restoration plans. We used information on habitat preferences of larvae (plant occurrence and water depth) and similarity in odonate community (Brooks et al. 2012) to select the most appropriate habitat and perform reintroduction and translocation. Moreover, since we aimed at expanding the population and ensuring future population growth we chose a habitat that has larger carrying capacity than the source population. Furthermore, the regular harvest technique of last instar larvae coupled with augmentation by egg transfer was a successful approach because it insured the growth of the host populations and had little effect on the population dynamics of the source population. Future restoration plans of extremely endangered species should take into account the sustainability of source population harvesting and use bioindicators in selecting new host habitats.

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