

**Does experimental marking of wings influence resighting success in *Mesamphiagrion laterale* and *Erythrodiplax umbrata*?
(Odonata: Coenagrionidae, Libellulidae)**

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Abstract. To investigate if experimental marking affects the probability of resighting, 1,610 individuals of *Mesamphiagrion laterale* (Selys, 1876) and 630 individuals of *Erythrodiplax umbrata* (Linnaeus, 1758) were marked at two sites in Colombia and analysed with respect to marking variables as follows: marking colour used, which wing was marked, and a combination, i.e. the interaction, of these two. The colour and the marked wing were varied, using a different wing each time, and red, black, blue, or green colour. The information was analyzed using contingency tables (Chi-square test) to compare the probability of resighting for individuals within a population marked with a specific colour or on a specific wing to the probability of resighting for all other individuals in the population. In *E. umbrata* the resighting probability ranged as follows: 90.1 to 95.7% (marking colour type), 90.4 to 95.2% (wing used), and 87.5 to 97.8% (wing-colour combination). In the case of *M. laterale*, the resighting probability ranged as follows: 57.2 to 65.0% (marking colour type), 58.8 to 65.6% (wing used), and 51.0 to 82.0% (wing-colour combination). The colour, wing, or combination of wing-colour used for marking didn't have an effect on the resighting probability, suggesting that the method and its variations are adequate to be used in mark-release-recapture studies on odonates. Recommendations are given on what has to be avoided to eliminate potential effects during the marking procedure.

Key words. Damselfly, dragonfly, Zygoptera, Anisoptera, South America, Colombia

Introduction

Numerous methods for sampling wild populations have been developed (reviewed by SOUTHWOOD & HENDERSON 2000). Of these, the technique of mark-release-recapture (MRR) has been widely used in demography and

behavioural studies in odonates (reviewed by CORDERO & STOKS 2008). The most common method used to mark dragonflies involves the use of fine point permanent ink felt pens to apply unique marks, such as several digit codes on one wing, thus enabling rapid field identification of individuals (WATANABE et al. 2004).

Marking could have a significant impact on behaviour and survival and hence recapture rates. For example, marked individuals might avoid areas that they associate with unpleasant experiences (e.g., marking *per se*). Damage caused to the individuals by the marking process might increase mortality thus reducing the chances of an individual being resighted (CORDERO & STOKS 2008). In mammals, birds, and amphibians, effects of marking on the behaviour of individuals have been reported (reviewed by ANDERSON et al. 2011). In odonates, adverse effects of marking have been demonstrated in several studies (BEUKEMA 2002; PARR & PARR 1979; BANKS & THOMPSON 1985; FINCKE 1986), with the disappearance rate of marked individuals increasing in the days after marking. Additionally, it has been found that handling during marking may impact negatively on mating success (GRETHER 1996), foraging success (GRETHER & GREY 1996), survival (GRETHER 1997), and territorial behaviour (ANDERSON & GRETHER 2010), thus creating a bias in the estimation of these parameters (ANDERSON et al. 2011).

The aim of this study is to evaluate if there is a measurable effect associated with marking a particular wing, colour used in marking, or wing-colour combination in the two species, *Mesamphiagrion laterale* and *Erythrodiplax umbrata*, in Colombia.

Material and methods

Study subjects and areas

Mesamphiagrion laterale (Selys, 1876) is a medium-sized coenagrionid damselfly endemic to the Eastern Cordillera (Colombia) and the Mérida Cordillera (Venezuela), at elevations between 750 and 2,850 m a.s.l. Mature adults show brown or black colours with light blue over postocular spots, thoracic stripes, the internal region of the femur and the dorsal region of S7–S9, their wings are hyaline, without bands. The marking of this species was conducted along a transect (50 m) in the Tominé impoundment, Guatavita municipality, at 04°56'N, 73°50'W and an elevation of 2,600 m a.s.l.,

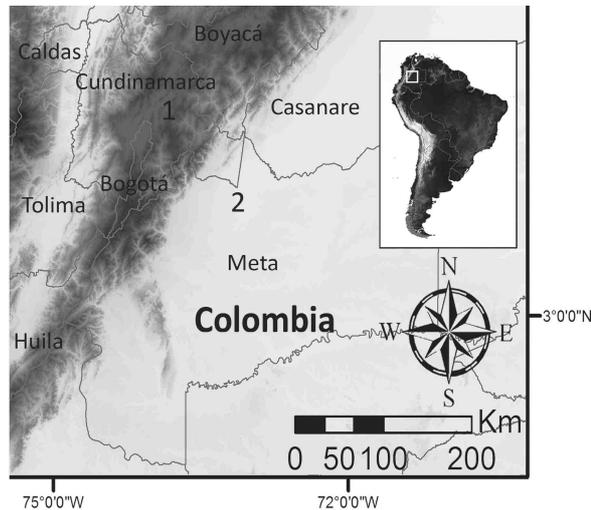
in the Cundinamarca department, Colombia (Fig. 1). The predominant vegetation in this area is *Acacia* sp. (Fabaceae), *Commelina* sp. (Commelinaceae), *Eichhornia crassipes* (Pontederiaceae), and *Brachiaria* sp. (Poaceae).

Erythrodiplax umbrata (Linnaeus, 1758) is a large libellulid dragonfly distributed from southern U.S.A. to northern Argentina. Males and females display two forms, one of which has a dark band in the four wings. In our study, the band was present only in males. The marking was conducted along a transect (200 m) inside a rice crop (*Oriza sativa* Linnaeus) in Pachaquiario, Puerto López, located at 04°05'N; and 75°53'W, at an elevation of 178 m a.s.l. in the Meta department, Colombia (Fig. 1).

Collection and marking

In this study 1,610 individuals of *M. laterale* and 630 individuals of *E. umbrata* were captured and marked. The wing marked and colour were chosen randomly. For *E. umbrata* we used three-digit codes from 001 to 630 that were applied in the prenodal region on one of their wings, thus avoiding the dark wing patch present in the males, while the *M. laterale* individuals were captured and marked in the postnodal region on one of their wings, using four-digit codes between 0001 and 1,610, with fine point makers Sharpie® in red, black, blue, or green colours. The colours were selected from the range

Figure 1. Situation of the study sites in Colombia. (1) Guatavita in the Cundinamarca department, where *Mesamphiagrion laterale* was studied; (2) Pachaquiario, Puerto López, in the Meta department, where *Erythrodiplax umbrata* was studied.



offered by the brand (www.sharpie.com). Only mature adults were marked, and handling did not last longer than one minute.

For both species, the order of wings and colours used for marking was pre-determined randomly following the procedure proposed by ANDERSON et al. (2011). Firstly, all possible colour (red, black, blue, or green) and wing (fore wing right-left; hind wing right-left) combinations were listed, secondly, the RAND function in Microsoft Excel[®] was used, and then, both columns were sorted by the random column command. In the case of *E. umbrata*, the field-work was conducted as follows: 21–24-vi-2010; 02–06-v-2011 and 10–29-iii-2012, from 09:00 to 17:00 h COT (= UTC-5). For *M. laterale*, field work was conducted in 2013 on the following days: 17-, 19-, 21-, 24-, 25-, and 28-iii; 02-, 03-, 05-, 07-, 09-, 12-, 15-, 23- and 29-iv; 02-, 06- and 27-v; 01- and 04-vi-2013, from 09:00 to 18:00 h COT. These dates included rainy and dry periods in each area. For both species, mark–release–recapture (MRR) was performed on each date. The procedure and the variations used for marking were consistent with MRR model assumptions proposed by ARNANSON et al. (1998) and adapted by CORDERO & STOKS (2008).

Statistical Analysis

To work with an inadequate sample might affect the significance of the results. For these reason, the total number of individuals needed to develop our analysis was estimated using the equation for data which the main variable is qualitative in finite populations (BADII 2011). The usage of this equation required knowing the population size of each species to determine the sample size needed for statistical analysis (confidence level: 95 %).

According to previous studies (e.g., HAGAN & REED 1988; ANDERSON et al. 2011) contingency tables (Pearson chi-square tests) were used to compare the resighting probability of marked individuals with the resighting probability for the rest of the individuals of the population, using the marking variations described. The analyses were done with the software Statistica 8.0 (www.statsoft.com).

Results

In the case of *Mesamphiagrion laterale*, 1,610 individuals (1,229 males, 381 females) were marked. The resighting probability, related to a colour, wing,

or combination colour-wing showed the following ranges: 57.2 to 65 % (colour), 58.8 to 65.6 % (wing), and 51.0 to 82.0 % (wing-colour combination). In *Erythrodiplax umbrata*, 630 individuals (106 males, 524 females) were marked, the resighting probability, by a colour, wing, or combination colour-wing, was in the following range: 90.1 to 95.7 % (colour), 90.4 to 95.2 % (wing), and 87.5 to 97.8 % (wing-colour combination). According to Pearson chi square tests (Tabs 1 and 2), for none of the species, the colour, wing (Tab. 1) or wing-colour combination (Tab. 2) used for marking were associated to the resighting probability. The number of individuals marked here is adequate to perform the statistics tests, since the results from the equation for data where the main variable is qualitative in finite populations, which estimated only *M. laterale* 269 individuals were required out of the 1,610 individuals that were marked, while 167 individuals of *E. umbrata* were necessary out of 630 individuals marked of this species.

The sex ratio in the recapture rate showed bias towards *M. laterale* males (3:1), and towards the *E. umbrata* females (1:5), however, there was no significant difference among sexes for both species ($p = 0.30$ and $p = 0.98$, respectively).

Discussion

BEIRINCKX et al. (2006) and CORDERO & STOKS (2008) found that in odonates the likelihood of recapture is higher for males, which could be relevant to evaluate the effect of marking. Even though the sex ratio showed bias towards *M. laterale* males (3:1) and towards *E. umbrata* females (1:5), there was no significant difference in the recapture rate among sexes for species in our study.

According to MALLET et al. (1987), handling during the marking procedure could increase mortality or induce dispersive behaviour, resulting in a decreasing likelihood of recapturing marked individuals. CORDERO (1994) found that the immediate mortality after marking is almost nil, so it is probable that the individuals leaving the reproductive sites are stressed by the handling during this procedure.

MANLY (1971) evaluated the effect of wing marking in the marking-recapture data of *Enallagma cyathigerum* (Charpentier, 1840), concluding that there was no negative effect from the marking over the total of the sam-

Table 1. Resighting probabilities of *Mesamphiagrion laterale* and *Erythrodiplax umbrata* in this study according to colour type of mark and marked wing. N – number of individuals; RC – number of individuals recaptured; PR – probability of resighting; FW – fore wing; HW – hind wing. *P*-values are from χ^2 -contingency tests. The probability of resighting according to the contingency tests is given in parentheses.

Colour or wing	<i>M. laterale</i>				Colour or Wing	<i>E. umbrata</i>			
	N	RC	PR	<i>P</i>		N	RC	PR	<i>P</i>
Black (90.2 %)	368	227	0.6	0.2	Black (61.9 %)	174	19	0.1	0.05
No black	1,242	799	0.6		No black	456	28	0.06	
Blue (94.8 %)	509	273	0.5	0.1	Blue (65.1 %)	183	10	0.05	0.2
No blue	1,101	744	0.6		No blue	447	37	0.08	
Green (95.7 %)	373	254	0.6	0.1	Green (59.5 %)	135	6	0.04	0.3
No green	1,237	763	0.6		No green	495	41	0.08	
Red (92.0 %)	364	272	0.7	0.1	Red (57.2 %)	138	12	0.08	0.1
No red	1,246	745	0.6		No red	492	35	0.07	
FW right (93.6 %)	398	279	0.7	0.1	FW right (58.8 %)	160	11	0.07	0.2
No FW right	1,212	733	0.6		No FW right	470	36	0.07	
FW left (93.3 %)	378	262	0.7	0.1	FW left (59.1 %)	152	11	0.07	0.2
No FW left	1,232	750	0.6		No FW left	478	36	0.07	
HW right (95.2 %)	528	277	0.5	0.1	HW right (65.6 %)	158	8	0.05	0.2
No HW right	1,082	735	0.6		No HW right	472	39	0.08	
HW left (90.4 %)	311	194	0.6	0.2	HW left (61.6 %)	160	17	0.1	0.07
No HW left	1,299	818	0.6		No HW left	470	30	0.06	

ple studied. Nonetheless, MANLY (1971) found a type of temporal marking effect, which reduces the survival of the organisms, specifically in the first days after marking has begun. Although he did not explain the possible causes leading to this effect, we infer that this might be a consequence of a “learning curve” during which the investigator is gaining experience in manipulating the organisms carefully.

Although MANLY (1971) did not find an effect of marking over the whole sample, other authors have reported the effect of this technique (PARR & PARR 1979; BANKS & THOMPSON 1985; FINCKE 1986) in studies with other odonate species. HANNON & HAFERNIK (2007) explained that the effect of marking in their study could be a consequence of two situations: Firstly, prolonged han-

Table 2. Resighting probabilities of *Mesamphiagrion laterale* and *Erythrodiplax umbrata* in this study for marked wing-colour type. N – number of individuals; RC – number of individuals recaptured; PR – probability of resighting; FW – fore wing; HW – hind wing. *P*-values are from χ^2 -contingency tests. The probability of resighting according to the contingency tests is given in parentheses.

Wing-colour combination	<i>M. laterale</i>				Wing-colour combination	<i>E. umbrata</i>			
	N	RC	PR	<i>P</i>		N	RC	PR	<i>P</i>
FW right red (94.6 %)	100	88	0.8	0.4	FW right red (53.2 %)	35	2	0.05	0.4
No FW right red	1,510	937	0.6		No FW right red	595	47	0.07	
FW left red (89.2 %)	71	56	0.7	0.4	FW left red (55.9 %)	33	4	0.1	0.3
No FW left red	1,539	969	0.6		No FW left red	597	45	0.07	
HW right red (94.3 %)	135	84	0.6	0.4	HW right red (61.6 %)	33	2	0.06	0.4
No HW right red	1,475	941	0.6		No HW right red	597	47	0.07	
HW left red (91.2 %)	62	43	0.7	0.4	HW left red (59.1 %)	31	3	0.09	0.3
No HW left red	1,548	982	0.6		No HW left red	599	46	0.07	
FW right black (87.8 %)	75	55	0.7	0.4	FW right black (57.7 %)	36	5	0.13	0.3
No FW right black	1,535	970	0.6		No FW right black	595	44	0.07	
FW left black (88.0 %)	91	87	0.9	0.4	FW left black (51.1 %)	44	6	0.13	0.3
No FW left black	1,519	938	0.6		No FW left black	586	43	0.07	
HW right black (92.0 %)	141	31	0.2	0.4	HW right black (82.0 %)	46	4	0.08	0.3
No HW right black	1,469	986	0.6		No HW right black	584	45	0.3	
HW left black (87.5 %)	59	45	0.7	0.4	HW left black (56.7 %)	49	7	0.3	0.3
No HW left black	1,551	980	0.6		No HW left black	581	42	0.07	
FW right blue (94.7 %)	113	72	0.6	0.4	FW right blue (61.1 %)	54	3	0.05	0.3
No FW right blue	1,497	953	0.6		No FW right blue	576	46	0.08	
FW left blue (91.5 %)	93	38	0.4	0.4	FW left blue (71.0 %)	43	4	0.09	0.3
No FW left blue	1,517	987	0.6		FW left blue	587	45	0.07	
HW right blue (97.8 %)	126	65	0.5	0.4	HW right blue (66.0 %)	44	1	0.02	0.4
No HW right blue	1,484	960	0.6		No HW right blue	586	48	0.08	
HW left blue (93.9 %)	174	95	0.5	0.4	HW left blue (64.7 %)	46	3	0.06	0.3
No HW left blue	1,436	930	0.6		No HW left blue	584	46	0.07	
FW right green (97.8 %)	110	64	0.5	0.4	FW right green (63.2 %)	33	0	0	0.4
No FW right green	1,500	961	0.6		No FW right green	597	49	0.08	
FW left green (94.3 %)	123	81	0.6	0.4	FW left green (60.3 %)	33	2	0.06	0.4
No FW left green	1,487	944	0.6		No FW left green	597	47	0.07	
HW right green (94.4 %)	120	97	0.8	0.4	HW right green (55.3 %)	34	2	0.05	0.4
No HW right green	1,490	928	0.6		No HW right green	596	47	0.07	
HW left green (94.7 %)	16	11	0.6	0.4	HW left green (59.3 %)	36	2	0.05	0.4
No HW left green	1,594	1,014	0.6		No HW left green	594	47	0.08	

dling time between marking and release, and secondly that the release did not occur in the capture site. MICHIELS & DHONDT (1989) did not find a marking effect, but they reported of damage on the wings of old individuals due to marking. UÉDA & IWASAKI (1982), NOMAKUCHI et al. (1988), and FINCKE (1988) found an increase in mortality due to handling during marking, probably as a consequence of the fact that in these studies several individuals were marked in the teneral state, in which they are susceptible to suffer damages because their integument is not hardened yet. In a population study (FPR & NACS unpubl. data) with *Euthore fasciata* (Hagen in Selys, 1853), we found that substances as liquid paper[®], a correction fluid, produced a negative effect, possibly as a result of the increase in the weight of the organism.

In this study, we avoided using those methods discussed above which resulted in harm to the insects and recommend when conducting MRR on Odonata, to write unique codes of several digits on any of the wings with fine point and permanent ink markers in the colours used in this study. We also make the following recommendations:

- i) Test the marking method before the beginning of the study, in order to practice the handling of the organisms and to corroborate that the method is effective to obtain the required data.
- ii) Mark with permanent ink so the mark will endure until an individual's death.
- iii) Do the marks with fine point markers just once; to re-write the codes might break or damage the wing, which will affect the behaviour and survival rate of an individual.
- iv) The marks must be precise and easy to see; their rapid detection with binoculars, or other means, helps to reduce the manipulation of individuals during recapture.
- v) Avoid handling individuals during marking for an extended period of time.
- vi) The release must occur at the capture site.
- vii) Do not mark individuals in a teneral state; they might be damaged and the marks can be erased.
- viii) Do not use substances for marking that might increase the weight of an individual and, as a consequence, its behaviour and chance of survival.

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