Behaviour and ecology of *Hemiphlebia mirabilis* (Odonata: Hemiphlebiidae)

Flicking display by a female *Hemiphlebia mirabilis*. Ming Ming Swamp, Grampians National Park.

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1 Introduction

The ancient greenling, *Hemiphlebia mirabilis*, is an endemic species of damselfly known from some wetlands in Victoria and Tasmania. In the 1970s this species was considered extinct or almost extinct (New 2007), and no populations were known by 1980 (Trueman et al. 1992). Populations were extirpated by habitat degradation, mainly due to drainage, agriculture and the effects of intense cattle breeding (New 2007). In fact, Davies (1985) declared that "the cow must be *Hemiphlebia*'s worst enemy". This species is of particular interest, not only due to concerns about its conservation, but especially because it is a taxonomic rarity, the only living member of its superfamily (Fraser 1955; Davies 1985). Some authors considered *Hemiphlebia* the sister group of all remaining Odonates (Fraser 1957), or all Lestoidea (Dijkstra et al. 2014). It has several unique characters on wing venation and also in larvae and spermatozoa (Trueman 1999), although it is unclear whether these represent ancestral or derived features.

The body colour is metallic green (sometimes bronze), and the size of the animal is so small (about 24 mm, Sant & New 1988; less than 25 mm, Watson 1995) that it is difficult to detect in the vegetation where it usually perches (Tillyard 1913). Nevertheless, both sexes show a remarkable "abdomen-flicking", which makes them highly conspicuous due to the whitish (and light bluish; see Results) coloration of the inferior abdominal appendages, which are expanded and displayed frequently. Sant & New (1988) describe in detail this abdominal display and adult perching behaviour, as well as several aspects of its larval biology.

Very little is known about the reproductive behaviour of *H. mirabilis*. Tillyard (1913) briefly described one copulation he witnessed, and proposed that the abdomen flicking was part of the courtship display. Fraser (1955) indicates that the collector who sent specimens to him (Roderick Dobson) observed that females also performed the abdominal display "though to a lesser extent", implying that this might not be part of the courtship. Sant and New (1988) observed only one mating during their field work, but give little details about it. Some authors state that they have not observed copulatory behaviour (Davies 1985; Richter 2009). To my knowledge, no other descriptions of reproductive behaviour of this species have been published. The most surprising fact is that apparently nobody has observed female *Hemiphlebia* laying eggs (Davies 1985).

Very recently, *H. mirabilis* range has been greatly increased by the discovery of several colonies in Western Victoria, one of which at Discovery Bay Coastal Park, near Nelson, seemed promising for a behavioural study (Richter 2009), given the high density of the species there (Richard Rowe, pers. comm.). Reiner Richter has subsequently observed copulatory behaviour and discovered new populations in Grampians National Park (Reiner Richter, pers. comm.), increasing again the geographical range of the species.

Odonates are well known model species for studies of sexual selection and evolutionary biology in general (Córdoba-Aguilar 2008). Their reproductive behaviour is unique, because males and females have two points of contact when mating, and also because males are able to remove or displace sperm from previous matings using their specialised genitalia (Waage 1979). In a review of postcopulatory sexual selection in this group, a taxonomic bias was found: almost all data have been obtained from a small number of species from the Coenagrionidae, Calopterygidae and Lestidae, and I suggested that *Hemiphlebia*, given its unique characters, should be a priority taxon in this context (Cordero Rivera & Córdoba-Aguilar 2010).

The goals of this study were therefore two fold. First, I wanted to observe and describe the reproductive behaviour and collect some specimens for a detailed morphological study of genitalia. Fraser (1955) described and figured the male intromittent organ, with two flagellae similar to that of other species known to use these structures to remove sperm (Córdoba-Aguilar & Cordero Rivera 2008). However, female internal organs remain unknown. Given that these structures are the "arena" where postcopulatory sexual selection takes place, their study is crucial. If *H. mirabilis* males displace sperm, this would suggest that sperm displacement and the dual function of male genitalia (Waage 1979) is a very old character in Odonates. The second goal was to develop captive breeding, which would allow obtaining enough specimens for a study of reproductive behaviour under controlled conditions in the laboratory. Given previous experiences by other researchers with this species, it seemed unlikely that reproduction would be observed, and therefore it might be not possible to obtain eggs and rear the species in captivity.

2 Methods

The study was done at Long Swamp (Figure 1), in the Discovery Bay Coastal Park, near to Nelson (Victoria) (Richter 2009), between 17 November and 7 December 2013. The first day I explored several areas of the swamp, and immediately found the first specimens of *Hemiphlebia*, which were conspicuous thanks to their abdominal curling display.

From 19 November to 7 December I visited daily (excluding rainy days) a spot of the swamp, where *Hemiphlebia* was common (coordinates: 38.110375ºS, 141.137474ºE, WGS84 datum). This place was selected because it was easily accessible from the road, and constituted a small favourable habitat connected to the big swamp only by its north side, allowing the study of a small fraction of the population (Figures 2 and 3). Using an image analysis software (KLONK 13.2, http://www.imagemeasurement.com/) I estimated that the area sampled was about 490 m^2 (Figure 3).

I decided to start a mark-recapture experiment to estimate survival rate and population density, and marking in the open swamp would be inefficient given the apparently huge size of the population (Fig. 1). Having a marked population is a convenient fact for demographical and behavioural studies (Cordero Rivera & Stoks 2008), because individually marked specimens are best for focal observations. Furthermore, concentrating observations in a small area allowed minimizing damage to vegetation, and I tried to use some already present tracks (Figure 4), apparently made by kangaroos, to further minimize the impact of my presence in the habitat.

Figure 1. A view of Long Swamp, showing the reed vegetation preferred by *H. mirabilis*.

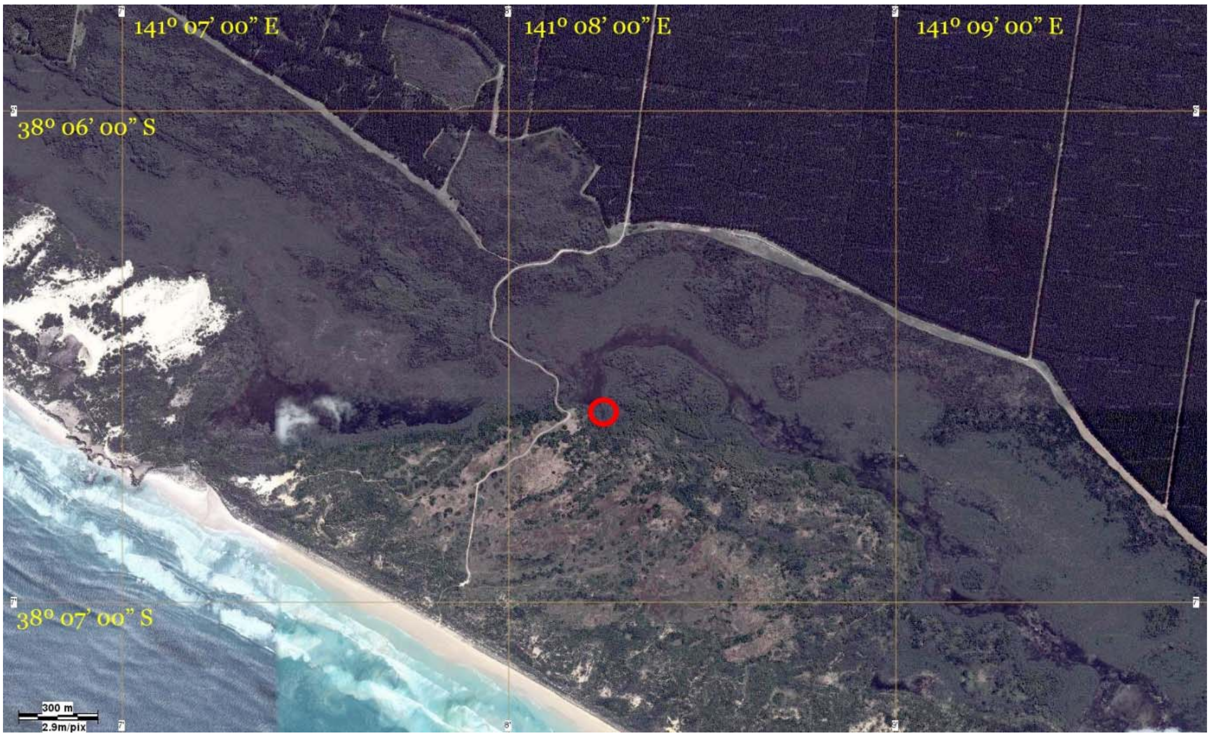

Figure 2. GoogleEarth image of Long Swamp (Nelson), showing the area where the study was conducted (red circle).

Figure 3. Detail of the study area in an image of GoogleEarth. The area sampled has a surface of about 490 m^2 and a perimeter of 123 m.

No observations were done on days 20 to 23 November and 5 December due to cold and rainy weather. I visited Ming Ming Swamp (Grampians National Park) on days 2 and 4 December, and *Hemiphlebia* was found abundant there also. Between 9 and 12 December I sampled some streams in NE Victoria, near Wodonga.

Marking was done with a permanent black ink pen (Faber-Castell Multimark 1525 S) on the external side of the right hind wing (Figure 5). At the moment of marking all individuals were sexed, examined for ectoparasites, their age recorded (as teneral, young or mature), and a subset were measured from the head to the tip of the abdomen (including appendages) and then released. Every sampling day, several mark-recapture sessions were completed, and recaptured specimens were recorded.

Figure 4. A view of the sector where all observations were done. Note the trail, which was already present when field work started, but that was enlarged by my presence.

Figure 5. Two marked specimens mating in my hand inside the insectary (see Methods).

To describe daily activity and reproductive behaviour I did 10-minute focal observations of undisturbed specimens, from 9 to 19 h, on days with suitable weather conditions (see below). The goal was to know when copulation takes place, and especially at which time of the day females perform oviposition. If the focal individual was unmarked, I tried to capture and mark it at the end of the 10-minute period. Overall, I observed 83 males and 79 females, and 33 remained unmarked because could not be captured. Nevertheless, given the high population size it is unlikely that the same specimen has been observed twice, because I moved between consecutive observation places. For statistical analyses, I assume that all observations belong to different specimens. Two focal males and one female were found to be teneral, and were excluded from the analyses (teneral individuals showed almost no activity). I recorded the time of observation, number of flicking movements, number of flights shorter than 50 cm and longer than 50 cm, the number of "rotations" (see below), the number of feeding flights, and the number of reproductive interactions (tandem attempts with other individuals, as the actor (for males) or as the receiver of the behaviour (for both sexes). Other behaviours less frequently observed (eye cleaning, abdomen grooming) were also recorded, but cannot be statistically analysed.

Copulatory behaviour was rarely observed. For instance, I observed one mating on 19 and 25 November and two on 26 November. Therefore, I decided to try to increase inter-individual encounters by using a mosquito net as an outdoor insectary, where a set of marked specimens was introduced (Figure 6). The insectary was used to elicit mating behaviour on days 29, 30 November and 1 and 3 December, between 11 and 16-17 h. At the end of the observations, the insectary was removed and individuals released. I observed 8 copulations outside and 20 inside the insectary, and 11 were interrupted at different times, to study sperm competition. The pair, or sometimes only the female, was immediately preserved in 70% ethanol for further analyses.

One thermometer was situated on the shade at about 1.5 m over water. Temperature was recorded approximately once every hour. Water temperature was measured on 25 November. Another thermometer was placed inside the insectary, but in this case sometimes it received direct sunlight, and was used to have a more biologically realistic temperature measurement, because mating individuals were under direct sunlight also.

Data of recapture histories of marked specimens (see Figures 5, 6 and 17 for examples of marked specimens) were analysed with the methods of Jolly (1965) and Manly & Parr (1968) implemented in POPAN5 to obtain population size estimates (Arnason et al. 1998). To estimate survival rate I used the information-theory approach implemented in MARK 6.2 (Cooch & White 2007), which is based on the Akaike Information Criterion (AIC) (Burnham & Anderson 1998). Teneral individuals were not included in the dataset, given their low numbers, and age (young/mature) was not considered in the analyses because the number of specimens marked young was low. I first explored the adjustment to the data of a model including two groups (males and females) (g) and time-dependence (t), model Phi(g*t) p(g*t) in the notation of Lebreton et al. (1992). The asterisks indicate that group and time interact (i.e., the model allowed different survival and recapture rates for both sexes all over the time). The goodness-of-fit tests implemented in MARK indicate that TEST 2, which is useful for testing the basic assumption of "equal catchability" of marked animals (Cooch & White 2007) was not significant (χ^2=28.57, df=37, p=0.838). TEST 3, which tests the assumption that all marked animals alive at (i) have the same probability of surviving to (i+1) was also not significant (χ^2=13.78, df=30, p=0.995). Overall, these two tests were not significant (χ^2=42.35, df=67, p=0.992). Therefore the time-dependent Cormak-Jolly-Seber model by groups (model Phi(g*t) p(g*t)) is a good starting model to describe the variability in the data. I run the program to select the best model as that which minimizes $QAIC_c$ (Quasi Akaike's Information Criterium corrected for overdispersion). I estimated the overdispersion parameter (c-hat) from this saturated model and divided this value by the c-hat estimated by the bootstrap procedure in MARK, and the resultant value (1.403707) was used to correct parameter estimates (Cooch & White 2007).

Mean values are presented with their standard error (SE) and sample size: mean±SE (N).

Figure 6. The mosquito net mounted on a corner of the swamp and a mating pair inside this insectary.

3 Results and Discussion

No individual was found with ectoparasites (mites). This contrast with the fact that at least two out of the very few specimens of *Ischnura heterosticta* found in the same habitat (6 males and 2 females) had at least one mite.

3.1 Body size

Part of the specimens captured for marking were measured to the nearest mm using callipers. Measurement error is likely to be high when handling alive animals, but allows screening phenotypic variability. This species is among the smallest zygopterans. At Long Swamp, mean body size (including anal appendages) was 25.1±0.05 (160) mm for males and 23.9± 0.06 (86) for females. Figure 7 shows the histogram of body size variation by sex. There was a negative relationship between body size and date of marking (males: Pearson r=-0.240, p=0.005; females: r=-0.195, p=0.045; Figure 8). This fact is common in odonates (Corbet 1999).

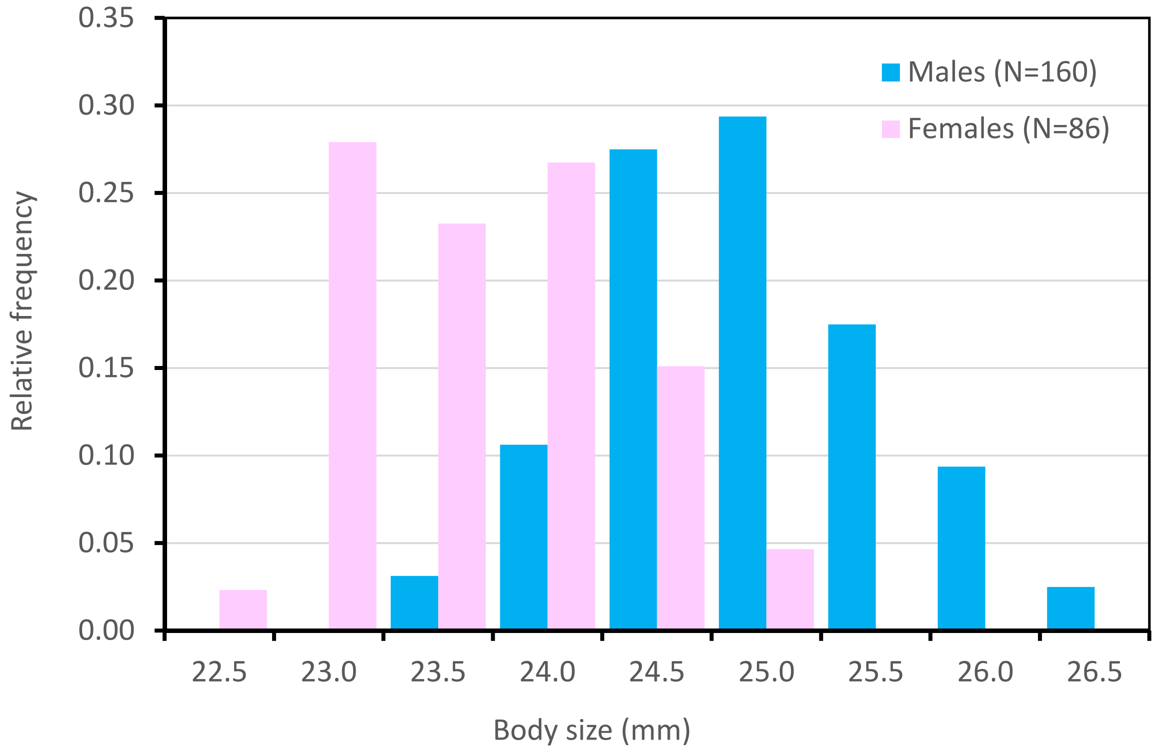

Figure 7. Histogram of body size variation for males and females of *H. mirabilis* at Long Swamp.

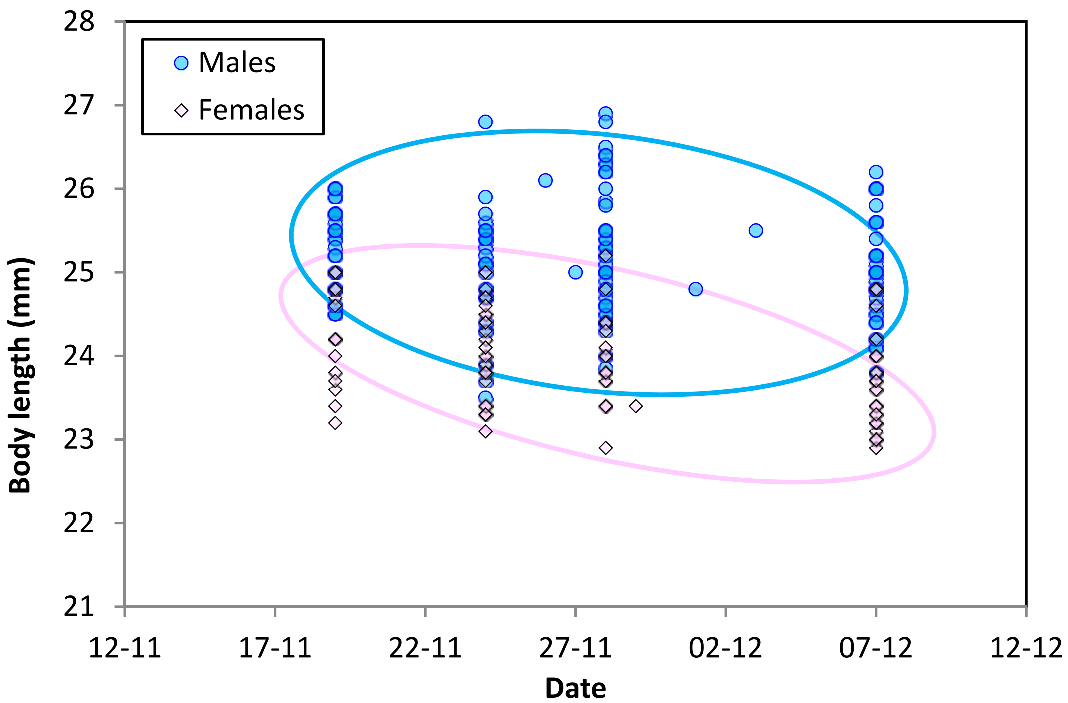

Figure 8. The relationship between date of marking and body length. In both sexes, animals marked later in the season were smaller. The ellipses show the predicted confidence intervals.

3.2 Population size and survivorship

Overall, I marked 598 males and 322 females, and recaptured 46 females (14.3%) and 168 males (28.1%). There were significant differences between sexes in apparent recapture rates (χ^2=24.07, p<0.001). Only 6 females were recaptured twice and 40 once. In the case of males, 133 were recaptured once, 29 twice and 6 three times. Of the 34 males and 18 females marked at the start of the study (19 November) none was recaptured the last day (7 December), but at least 5 males and one female marked on 24 November were still alive at the end of the field work. So maximum longevity is probable about three weeks.

Given the low recapture rates, my population size estimates have large standard errors. Figure 9 shows the estimated population size (±SE) obtained with the methods of Jolly and Manly-Parr. These estimates suggest that population size was about 800 males and 500 females in the middle of the study, and a number clearly lower at the end (estimated size for 6 December was 217±53 males and 166±95 females, average of both methods). This lower population size at the end of the fieldwork is the perception I had when I was searching for individuals for focal observations. Given that the study area was about 490 m^2 this means that density peaked at 2-3 individuals per square meter. My field observations are in agreement this this estimate.

The area of favourable habitat for *H. mirabilis* at Long Swamp is difficult to estimate. During field observations, the maximum density was observed at areas with 20-40 cm of water, which probably dry up in summer. *Hemiphlebia* perched on the dominant plant, a reed that might be *Baumea juncea* (Figure 10). Using Google Earth images, I roughly estimated the area with surface water at Long Swamp, which is the favourable habitat for *Hemiphlebia* as a minimum of 300,000 m^2. Taking

into account minimum and maximum population estimates, this means that *H. mirabilis* had a total population size oscillating between 174,000 to 1,274,000 individuals.

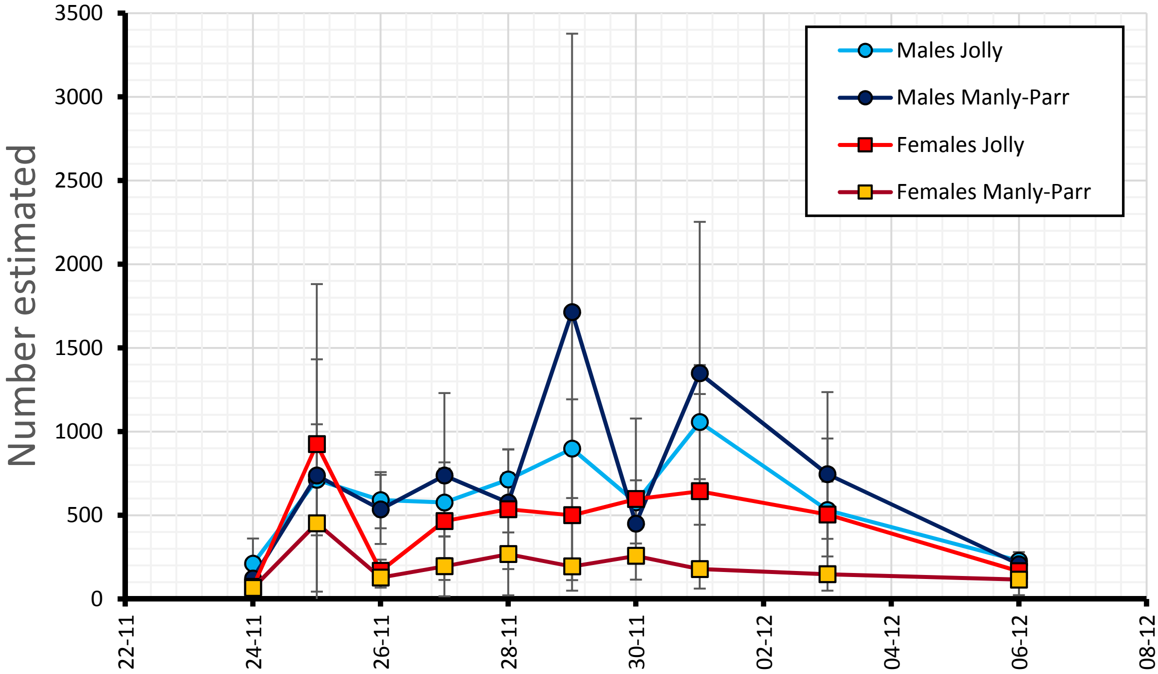

Figure 9. Population size estimates (±SE) for *Hemiphlebia mirabilis* at a sector of 490 m² of Long Swamp, in November and December 2013.

Figure 10. The preferred microhabitat of *H. mirabilis* at Long Swamp. Likely *Baumea juncea*.

The results of model selection using MARK are presented in Table 1. The first model [Phi(g) p(t)] has a relative weight of 0.81864/0.07598=10.77 in relation to the second. This means that the statistical support for a model with two survival rates, one for each sex, and a single recapture rate for both sexes but variable each day, is the best describing the variability of the data. It has 11 times more support than the second model, which uses a common survival rate for both sexes and time-dependent recapture [Phi(.) p(t)].

Using model Phi(g) p(t), daily survival rate was estimated as 0.8714 (SE 0.0194) for males and 0.7960 (SE 0.0315) for females. These values are very similar to the values published for small non-territorial Coenagrionidae, revised in Cordero Rivera & Stoks (2008), which range 0.794-0.960 for males and 0.579-0.898 for females. In *Ischnura hastata*, a species with similar body size, survival rates have been estimated as 0.571 for males and 0.557 for females (Lorenzo-Carballa et al, unpublished).

Daily survival rates translate into longevity using the formula of Cook et al (1967):

$$\textbf{Longevity} = -1/\log_e(\textbf{survival})$$

Expected longevity for males is therefore 7.3 days and for females 4.4 days as adults (excluding the teneral phase). The mean observed lifespan for individuals recaptured at least once was 4.7±0.27 (168) for males and 4.4±0.47 (46) for females. Observed lifespan for individuals marked as young and recaptured at least once was 6.6±0.87 (18) for males and 3.9±0.92 (9) for females. One female was found dead on the water on the hottest day (27 November) and two individuals were found in spider webs.

Recapture rate was very variable, between 5% for 24 November and 26% for 6 December (Table 2), probably due to variable climatic conditions, but also to inconstant sampling effort, because I was involved in focal observations and experiments inside the insectary.

Table 1. Model selection for mature *Hemiphlebia mirabilis* in Long Swamp, Nov-Dec 2013. Model name uses the notation of Lebreton et al (1992). The results are corrected for c-hat= 1.403707.

Model	QAICc	Delta QAICc	AICc Weights	Model Likelihood	Num. Par	QDeviance
{Phi(g) p(t) PIM}	1330.6889	0	0.81864	1	13	291.3569
{Phi(.) p(t) PIM}	1335.4433	4.7544	0.07598	0.0928	12	298.1688
{Phi(.) p(g*t) PIM}	1335.6879	4.9990	0.06723	0.0821	23	275.5311
{Phi(g) p(g*t) PIM}	1337.7388	7.0499	0.02411	0.0295	24	275.4742
{Phi(t) p(g*t) PIM}	1340.2903	9.6014	0.00673	0.0082	32	260.9924
{Phi(t) p(t) PIM}	1341.5025	10.8136	0.00367	0.0045	21	285.5476
{Phi(.) p(g) PIM}	1342.5777	11.8888	0.00215	0.0026	3	323.6188
{Phi(g) p(g) PIM}	1344.5654	13.8765	0.00079	0.0010	4	323.5892
{Phi(t) p(g) PIM}	1346.3041	15.6152	0.00033	0.0004	13	306.9721
{Phi(g) p(.) PIM}	1347.0602	16.3713	0.00023	0.0003	3	328.1014
{Phi(g*t) p(t) PIM}	1348.4405	17.7516	0.00011	0.0001	32	269.1426
{Phi(.) p(.) PIM}	1352.1003	21.4114	0.00002	0	2	335.1545
{Phi(t) p(.) PIM}	1354.8799	24.1910	0	0	12	317.6054
{Phi(g*t) p(g*t) PIM}	1360.2434	29.5545	0	0	42	259.2200
{Phi(g*t) p(g) PIM}	1365.1306	34.4417	0	0	24	302.8659
{Phi(g*t) p(.) PIM}	1366.9164	36.2275	0	0	23	306.7596

Table 2. Real function parameters of {Phi(g) p(t) PIM}, standard error and confidence intervals corrected for c-hat= 1.4037068.

				95% Confidence interval	
		Estimate	Standard Error	Lower	Upper
Males	Phi	0.8714	0.0194	0.8283	0.9048
Females	Phi	0.7960	0.0315	0.7275	0.8509
19-11	p	0.2116	0.1208	0.0610	0.5258
24-11	p	0.0491	0.0256	0.0174	0.1312
25-11	p	0.1199	0.0306	0.0717	0.1938
26-11	p	0.0525	0.0185	0.0260	0.1030
27-11	p	0.1129	0.0268	0.0701	0.1771
28-11	p	0.0616	0.0181	0.0343	0.1083
29-11	p	0.1177	0.0251	0.0767	0.1764
30-11	p	0.1033	0.0231	0.0660	0.1580
01-12	p	0.1039	0.0254	0.0635	0.1653
03-12	p	0.1540	0.0401	0.0906	0.2495
06-12	p	0.2653	0.0527	0.1754	0.3802

3.3 Maturation

Adult *Hemiphlebia mirabilis* were observed perching on swamp vegetation during all the study period. The population was huge at the start of the study (see Figure 7) but most animals were still young.

Teneral (newly emerged) individuals have a metallic green coloration, pale eyes, and wings with a vitreous appearance (Figure 11). Their integument is unhardened, and have a weak flight. This condition seems to last for about 24 h, but only four teneral males were recaptured, and therefore data are scarce. One day after emergence wings become transparent, but the tegument is still unhardened. These animals are young and sexually immature. Four females and two males marked at this age were recaptured. Female 345 was marked as a young adult on 26 November and was observed mating on 29 November, at an estimated age of 5-6 days. Animals become progressively darker and their wings less flexible with age, as is usual in damselflies (Corbet 1999). Their body colour changes to bronze (Figure 12) when they are apparently about two weeks old, but again data are too scarce to estimate maturation with precision. Animals observed at Ming Ming Swamp (Grampians National Park) seemed more bronze than those of Long Swamp, even when they were clearly young. It might be possible that local factors affect colour change.

Mature adult males show bright gold-pearl coloration on the labrum and postclypeus (Figure 13), which is very reflective under direct sunlight. Furthermore, the lower anal appendages of males and the valves of the females are of a bright white colour, but in some specimens show a bluish tint when under direct sun (Figure 14). This sky bluish coloration has apparently not been noticed before, because descriptions seem based on dead specimens (for instance the detailed description of Fraser 1955), and appear only in fully mature individuals. Not all specimens seem to develop the bluish coloration.

Figure 11. A teneral male of *H. mirabilis*. Long Swamp, 27 November 2013. Note the vitreous wings, and the clear eyes.

Figure 12. Courtship by a green male on a bronze female. Bronze coloration appears when animals are rather old (probably two weeks) is seems more frequent in females. Note the dark eyes of the male (dorsally), and compare with Figure 11.

Figure 13. Fully mature males show a reflective coloration on the labrum and postclypeus, which is very conspicuous under the sun. In females this coloration is less bright.

Figure 14. The most conspicuous morphological character of *H. mirabilis* is the white anal appendages. These are bright white but change into sky blue in some specimens, like this male trying to get the female in tandem.

3.4 Daily activity

Adults are very reluctant or unable to fly at temperatures below 17-18ºC on windy days, but in some cases even reproductive activity was observed at about 15ºC, provided that there was no wind. Figure 15 shows the variability of air temperature measured at approximately 1.5 m over water on the shade of one shrub. Temperatures oscillated between 14.4 and 37.5ºC over the study period. On 27 November, air temperature increased from 22.5ºC at 9:13 h to 37.5ºC at 14:07. This extreme situation allowed observing the behaviour of *H. mirabilis* at very high temperatures. Animals disappeared from the swamp when temperatures were above 35ºC (at 13 h on 27 November). Careful searching allowed finding some individuals under the bushes, or perched on the base of vegetation at about 5 cm from the water. Temperature was 30.4ºC at this point, when it was 36.5ºC on the shade at 1.5m above water. Apparently, *H. mirabilis* is very sensitive to both, low and high temperatures.

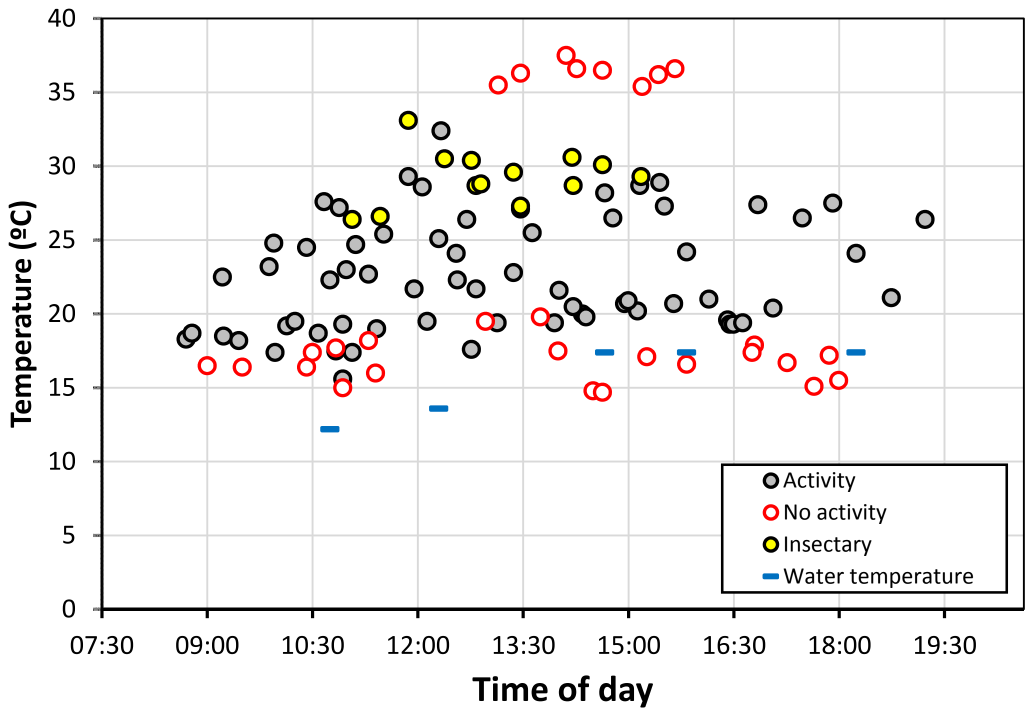

Figure 15. Variation in air temperature at the study site. Animals were active (flying, mating, capturing prey) at temperatures above 17-18ºC. On a single day (27 November) temperatures achieved over 35ºC in the middle of the day and animals became also inactive. Yellow dots indicate temperature measurements inside a mosquito net used as insectary. Water temperature was measured on 25 November.

Focal observations were done on randomly selected individuals, which were captured and marked (when possible) at the end of the 10 minutes of observation (see Methods). Observations started at about 9:00 h if animals were active, and ended at about 18:30-19h, except for periods of marking and experiments inside the insectary. After excluding three individuals that were teneral and showed no activity, sample sizes are 6-11 individuals of each sex per hour.

Adult behaviour surveyed included short flights (less than 50 cm), which were not directed to prey; long flights (more than 50 cm); feeding flights (very fast and short flights clearly directed to

a potential prey); the characteristic "abdomen flicking" (Figure 16); a sudden rotation movement on the perch, which was usually done to focus on other individuals and prey; and reproductive activities (for males this included attempts to get another individual in tandem, and the number of tandem attempts received from other males; for females only tandem attempts received). Other behaviours observed were eye cleaning and abdomen grooming, and, when two males were very close, a short face-off flight. These were observed at low frequency and are not analysed here.

Figure 16. Adominal flicking sequence of a female *H. mirabilis*. Ming Ming Swamp, Grampians National Park.

Hemiphlebia mirabilis spends most of its time perched, being a weak flier (New 1993). On 7 December, at the end of the study, I searched for marked animals in the main swamp, near the studied sector, but found none in a sample of 30 specimens. This suggests that they disperse very little. I collected legs from 30 males and 30 females from the Ming Ming Swamp for DNA analyses. A study of genetic differentiation between populations would be useful to know whether behavioural observations, which suggest limited dispersal ability, are confirmed.

Table 3 shows the mean values by sex for these behaviours. Males were slightly more active and made 3.3±0.3 (83) short flights versus 2.8±0.3 (79) for females over the 10 minutes period, but these differences are not significant (t_{157}=1.267, p=0.207). Long flights were very rare, and never more than four in 10 minutes in males and only one in females. The most conspicuous behaviour is abdominal flicking (Figure 16), which was done on average 22-25 times in 10 minutes, a similar rate by both sexes (Table 3). This behaviour was performed almost always after a flight (Tillyard 1913), when its intensity was higher (Sant & New 1988). Even if Tillyard (1913) suggested that females use abdominal flicking to attract males (and vice-versa), this could not be confirmed. Furthermore, the fact that both males and females perform this behaviour in absence of any reproductive interaction, suggests that this is ordinarily not a courtship display. Nevertheless, males do use

abdominal flicking in precopulatory courtship (see below). Both sexes performed the "high flick" abdominal display of Sant & New (1988), even if these authors reported it only for males.

Fast rotations over the perch were also very commonly observed (Richter 2009). In my experience, this behaviour is very rarely seen in other species of damselflies, but *H. mirabilis* performs on average one per minute in females and almost two per minute in males. In about one quarter of cases rotations were clearly directed to potential prey and were observed before a feeding flight. In further 10% of cases these rotations were directed to conspecifics, but in the remaining cases I could not detect the cause of this behaviour.

Reproductive activity was rare. Of the 79 females observed, only three copulated, and of the 83 males, only one copulated during the 10 minutes of focal observation. On average males are more involved in reproductive interactions than females (Table 3), because male-male tandem attempts were common. When two males interacted, sometimes a short face-off flight was observed, especially after one tandem attempt.

Table 3. Summary statistics for the behaviours observed in focal individuals. Values refer to 10 minutes of observation. Sample sizes are 81 males and 78 females (tenerals excluded), observed between 9 and 19 h.

Behaviour	Sex	Mean	SE	Maximum
Short flights (less than 50 cm)	males	3.321	0.289	12
	females	2.795	0.299	13
Long flights (more than 50 cm)	males	0.185	0.071	4
	females	0.064	0.028	1
Feeding flights	males	0.325	0.066	2
	females	0.487	0.087	3
Abdominal flicking	males	22.481	2.384	119
	females	25.192	3.941	172
Rotations	males	2.123	0.229	10
	females	2.064	0.286	17
Proportion of rotations directed to prey	males	0.211	0.041	1
	females	0.287	0.044	1
Proportion of rotations directed to a conspecific	males	0.100	0.036	1
	females	0.107	0.036	1
Reproductive interactions	males	0.309	0.065	2
	females	0.064	0.028	1

The abdominal flicking display peaked in the middle of the day, particularly in females (Figure 17). Nevertheless, males were actively searching for mates all over the day and females showed a similar activity (Figure 18). Females were difficult to find during the middle hours of the day, and sometimes I observed females perching down on the reeds, apparently to become less visible to males. Nevertheless, the very few matings and tandem attempts observed on females, were mainly done after 15 h (Figure 19). This apparent contradiction is likely due to the hiding behaviour of females during the central hours of the day.

The remaining two behaviours, rotation (Figure 20) and feeding flights (Figure 21) were observed at a similar rate over the day.

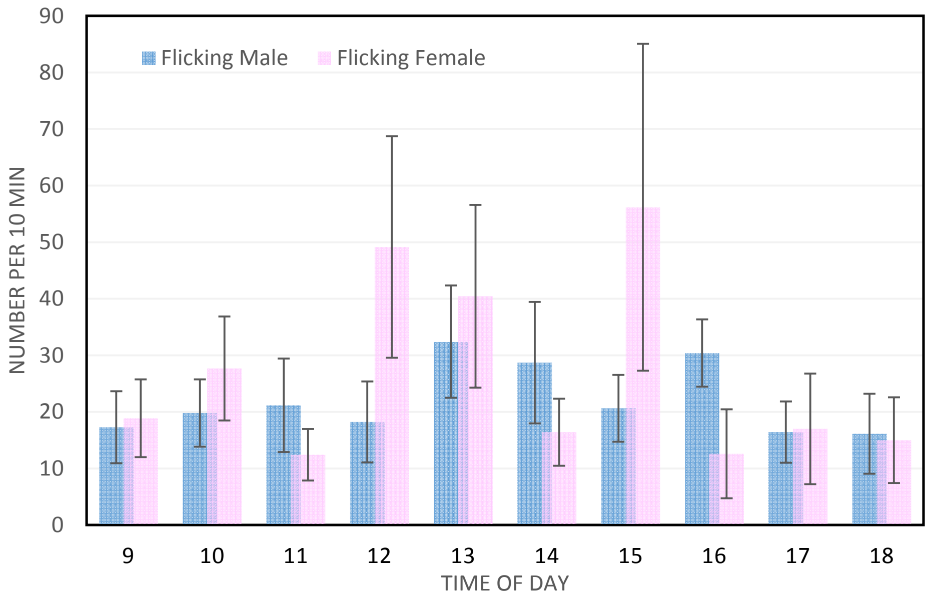

Figure 17. Daily variation in abdominal flicking display by males and females. Note that this behaviour was more frequent, particularly in females, between 12 and 15 h.

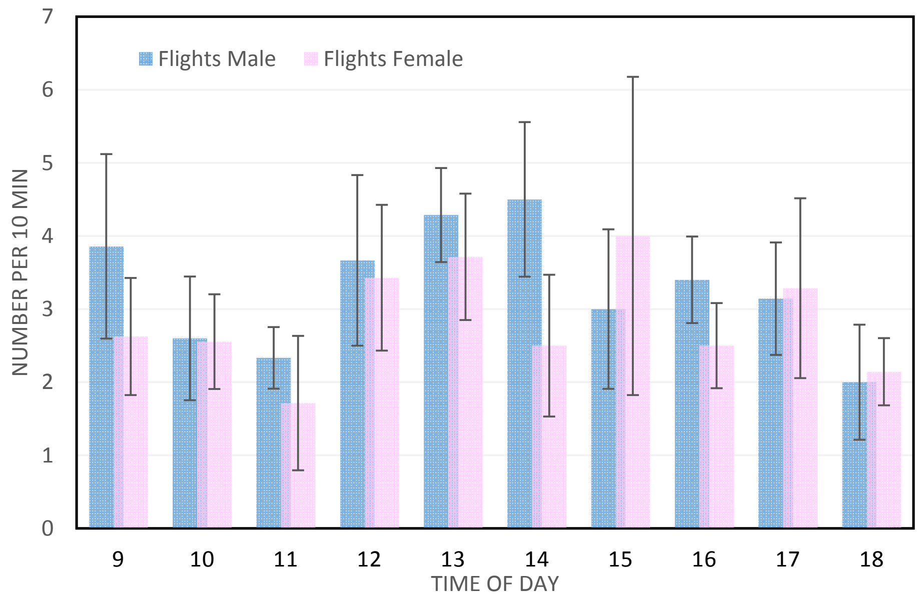

Figure 18. Daily variation in flight activity (short flights, less than 50 cm).

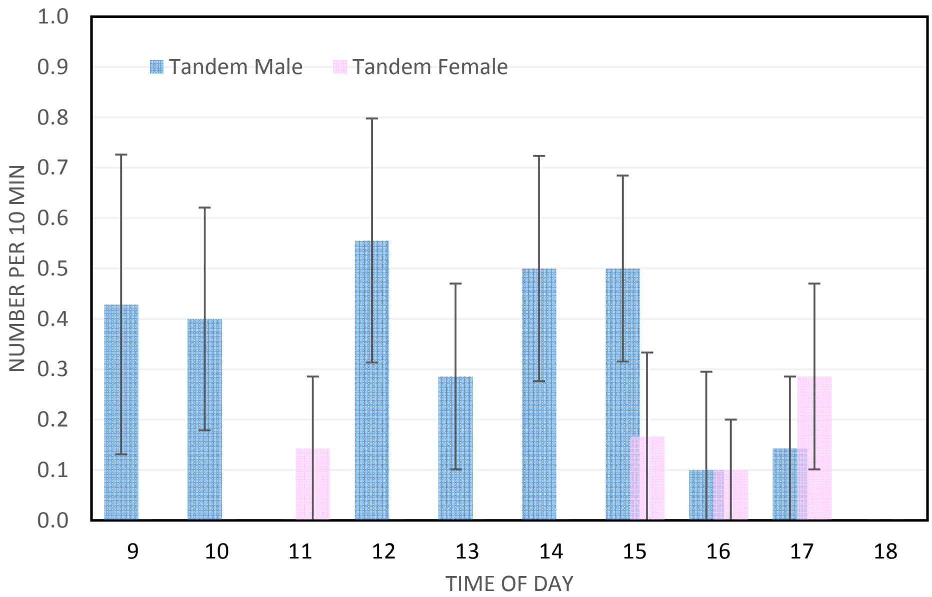

Figure 19. Variation in reproductive activity over the day (number of tandem interactions initiated or received per individual). Males were actively searching for mates during all the observation period. Surprisingly, females were more approached after 15 h, probably because in the central hours of the day they remained perched in concealed spots and were rarely detected by males.

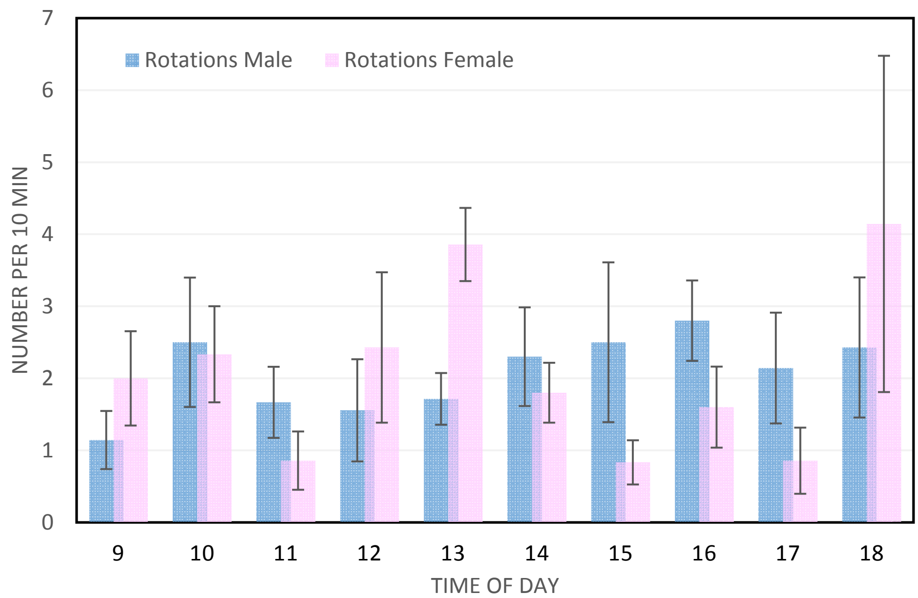

Figure 20. Daily variation in the number of "rotations" (sudden changes in the position of perched individuals). This behaviour was commonly observed when a potential prey or another conspecific was nearby.

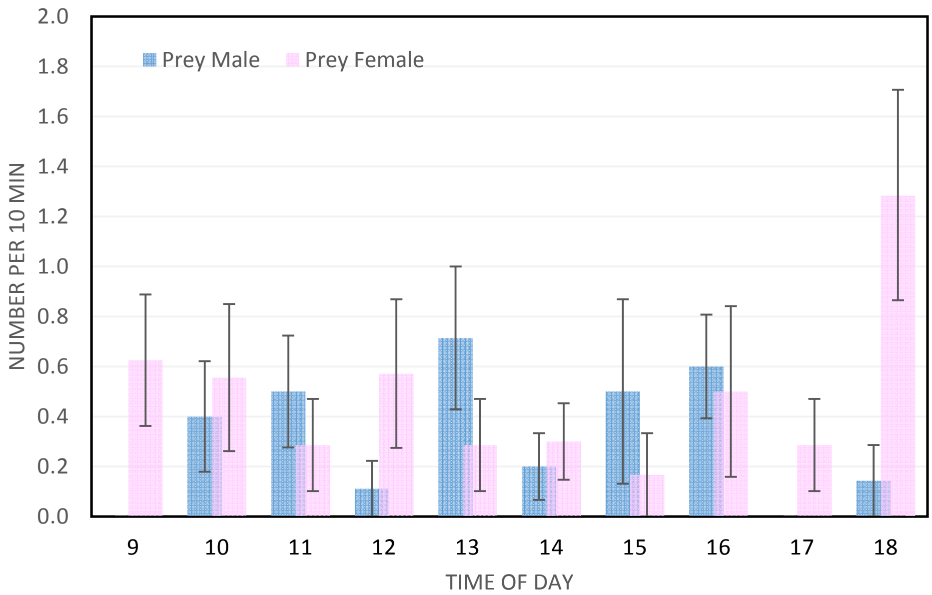

Figure 21. Feeding activity was observed all over the day, but peaked in late afternoon in females. Females were then commonly observed facing the sun, and making feeding flights and rotations to prey.

3.5 Reproductive behaviour

As mentioned above, mating frequency is surprisingly low in this species. Even if males and females are observed in large numbers, they seldom interact. Furthermore, I was unable to observe oviposition, as has happened to previous researchers (Sant & New 1988). Therefore, oviposition behaviour continues to be a mystery in *Hemiphlebia*.

There have been suggestions in the literature indicating that males use the curling of the abdomen to display and attract females (Tillyard 1913), and that females respond to male abdominal flicking by performing the same display (Davies 1985). My observations nevertheless do not confirm this. Both sexes perform abdominal flicking very frequently, particularly after flights, *even in the absence of conspecifics*. Females can perform this behaviour even more actively than males when they are alone and undisturbed (up to 172 times in 10 minutes compared to 119 in males, Table 3). Some other damselflies, particularly *Ischnura*, perform a similar abdominal display, but not so extreme, and especially without such upwards curling of the abdomen. In some cases both sexes displayed when another conspecific was close, but it seemed more a message to make them visible than a reproductive display. Experiments are needed to clarify this.

Over the study period, I observed 28 copulations, 20 of them inside the insectary. Mating behaviour is similar to Coenagrionidae, but with some peculiarities. When a male detects a potential partner he makes a fast flight, similar to feeding flights, and grasps the female by the wings (Fig. 22) with his legs. Occasionally males try to get in tandem other males, but this behaviour lasts usually only a few seconds. Some females curl their abdomen upwards when are immobilised by males, and try to dislodge the male, in a clear refusal behaviour (Fig. 23), which sometimes was successful. In the insectary, these tandems which did not end in copula lasted 8.4±2.4 (4) minutes. Receptive females remain motionless, and adopt a characteristic position with their abdomen

curved downwards in the junction between the first and second abdominal segment, and upwards between third and fourth segment. The position recalls a capital Z (Figs. 24 and 25). After a variable time motionless, males start to make their characteristic abdominal flicking, in a clear courtship behaviour (Fig. 25). Then, males curve their abdomen upwards and grasp the female's prothorax with their abdominal appendages. The average time between female capture and tandem formation was 6.20±1.17 (19) minutes, with a range of 1.67 to 23.0 minutes. When zygopterans are handled for marking, it is not rare that some loose one or more legs (Cordero Rivera et al. 2002). In the case of *H. mirabilis* this never happened, and all individuals had six legs when first captured. Furthermore, I found out that taking legs from adult *H. mirabilis* to sample tissues for DNA required the use of scissors instead of forceps, because forcibly removing one leg can produce damage on the animal, due to muscular tissues remaining attached to the leg. Given that legs are used to immobilise females during courtship, perhaps this explain why *H. mirabilis* males do not autotomize their legs.

Figure 22. Male *Hemiphlebia* use their legs to immobilise the female, and then perform courtship.

Figure 23. Female refusal behaviour, using the abdomen to try to dislodge the male.

Figure 24. Female acceptance of copulation is characterized by the "Z" position of her abdomen.

Figure 25. Courtship behaviour of male *H. mirabilis*. They use the "flicking" display characteristic of this species. Note the "Z" position of female abdomen, which signals receptivity. In some cases, males also curl their abdomen downwards during this display.

After tandem, males perform the sperm translocation (Fig. 26), which lasted on average 3.5±0.24 (26) seconds (range 1-6 sec). This behaviour always preceded copulation, contrarily to what was previously reported (Sant & New 1988). Copulatory movements are similar to Coenagrionidae, and had two clear phases, which correspond with stage I and II as described for *Enallagma cyathigerum* (Miller & Miller 1981). In all species so far studied, stage I is used to remove sperm from previous matings and stage II to inseminate (Córdoba-Aguilar et al. 2003). This is likely the case also for *H. mirabilis* (Figure 27). Sperm volume measurements will allow clarifying these behaviours. Dissections will be made on the 12 females whose copulation was interrupted at the end of stage I and the volume of sperm compared to the volume stored by 10 females captured at the end of copulation.

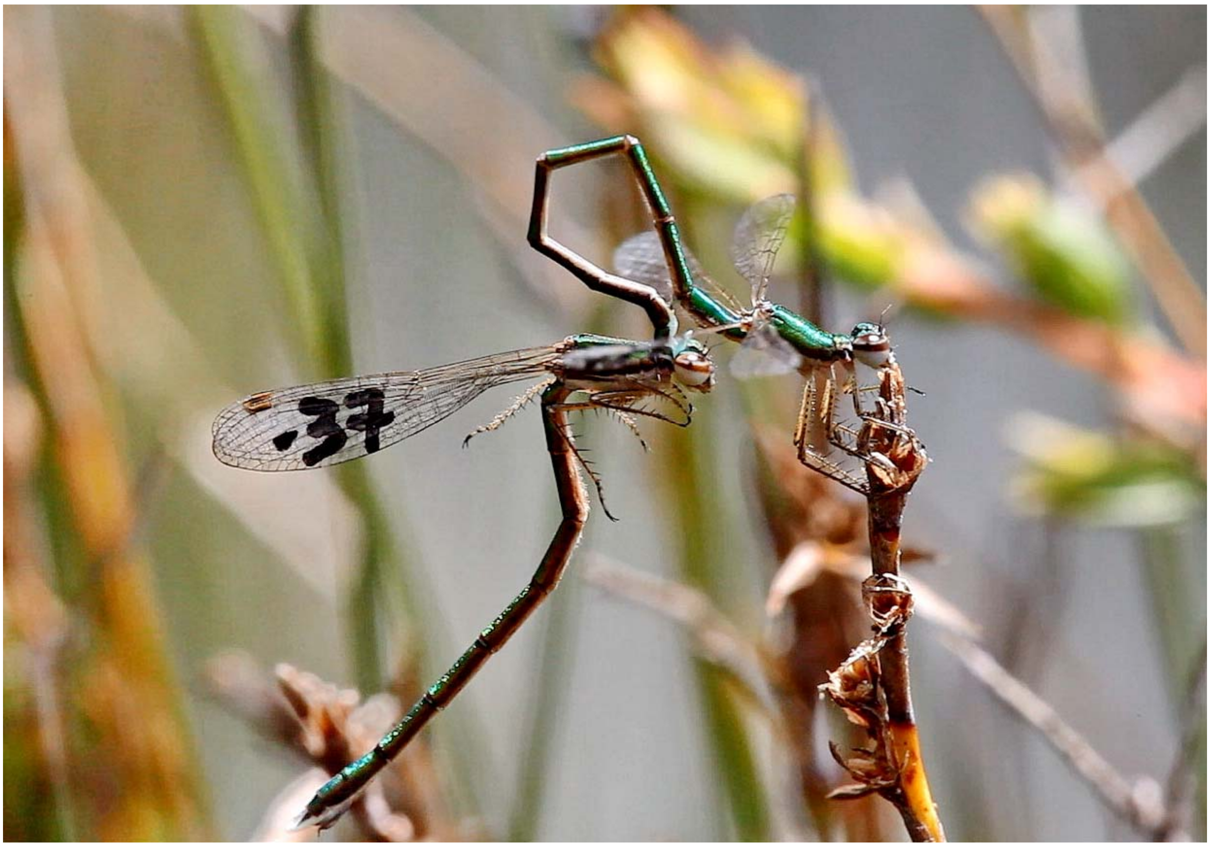

Figure 26. The intra-male sperm translocation behaviour. All matings observed (N=28) were preceded by this behaviour. Frame extracted from a video.

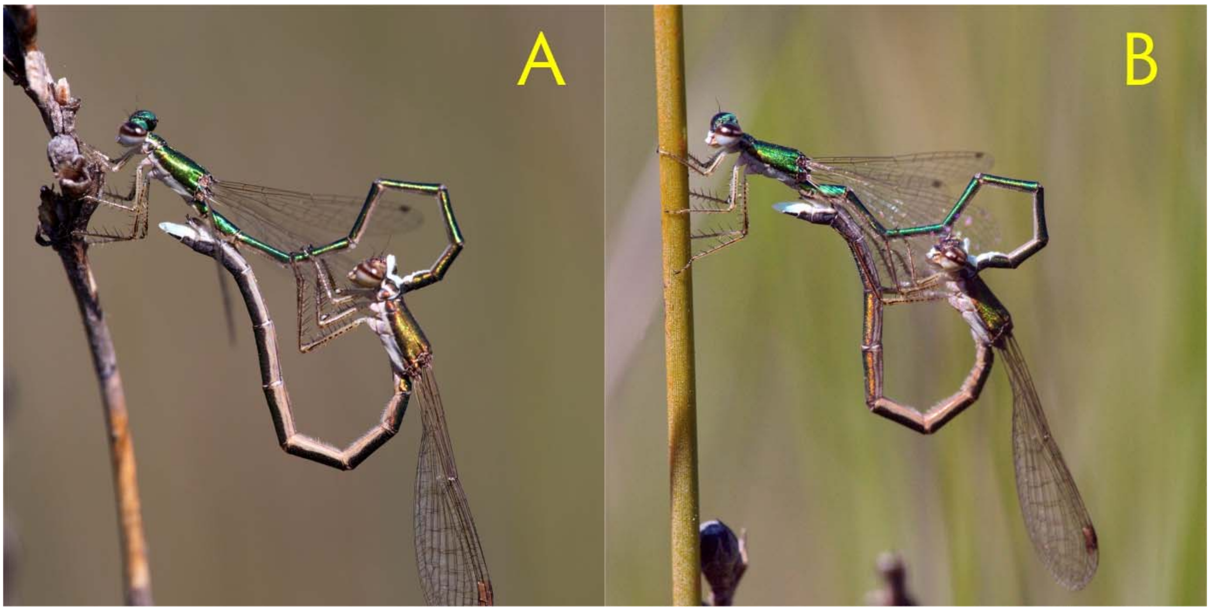

Figure 27. Copulatory movements in *H. mirabilis* match the description of stage I (A) and stage II (B) of Miller and Miller (1981). Stage I is involved in rivals' sperm removal and insemination takes place during stage II.

Copulation lasted 10.53±1.17 (14) minutes (range 4.1-18.7 min), and its duration is not related to time of day (Fig. 28) or air temperature (Fig. 29). Most of copulatory activity is due to stage I, which lasted 9.96±1.30 (12) minutes, as occurs in other damselfly species (Córdoba-Aguilar et al. 2003). Stage II is of short duration (1.08±0.11 (13)), and shows little variability. At the end of copulation, all males fly off immediately, but usually perched nearby. Females either fly (N=5) or remain perched for a short time (N=4). Given that I was interested in capturing some specimens for estimating sperm volumes, I could not dedicate time to follow mated females, but none showed signs of oviposition. One female could be observed for about 2 minutes after copula. She apparently did not expel sperm (Córdoba-Aguilar 2006), but made conspicuous movements of the external genitalia.

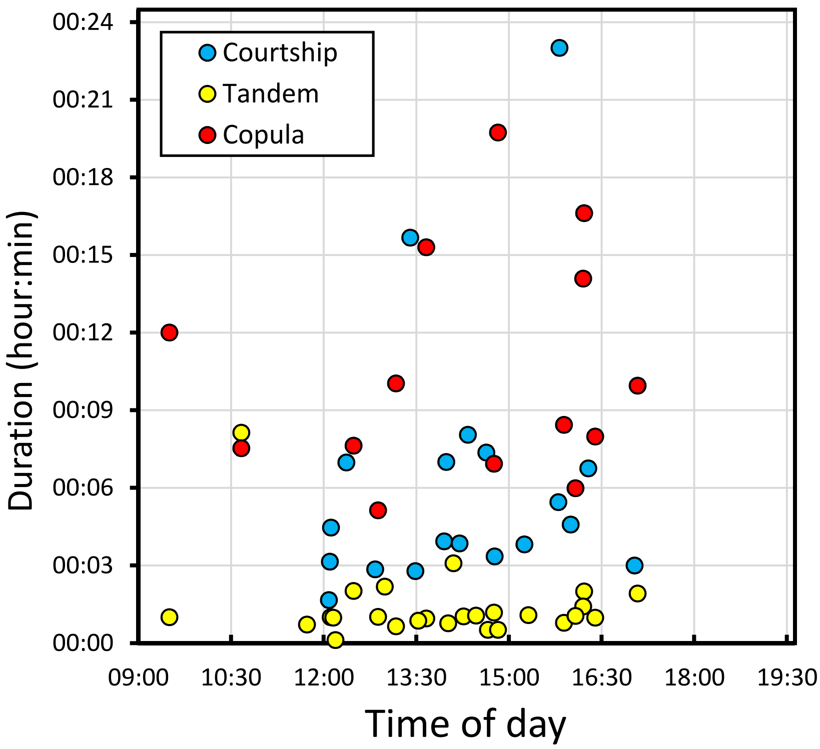

Figure 28. The relationship between time of day and duration of reproductive behaviours of *H. mirabilis*. Courtship refers to the time between female capture and tandem formation. Tandem indicates the time the pair remained in tandem before copulation. Finally, copulation refers to the time between the start and the end of the copulatory wheel.

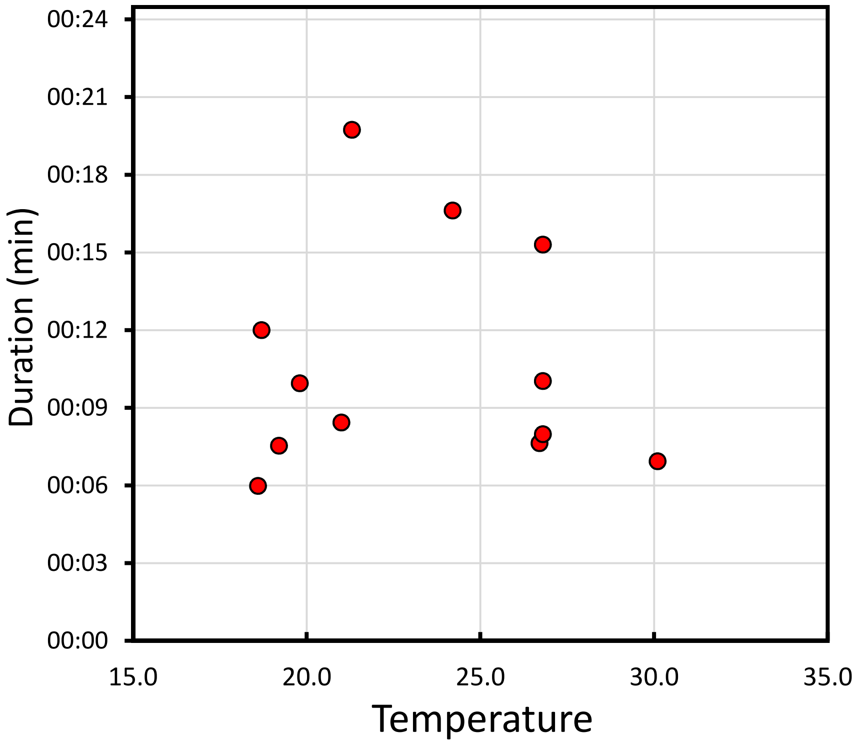

Figure 29. The relationship between air temperature and copulation duration in *H. mirabilis.*

I could not observe a single female laying eggs. It is clear that oviposition does not take place in tandem after mating, so it should be performed by females alone. As mentioned above none of the females observed during focal sampling lay eggs neither. On 25 November I collected 10 females apparently mature and put them in plastic containers with humid filter paper, which elicits oviposition behaviour in many zygopterans (Van Gossum et al. 2003). They were retained for two hours on a shaded spot, and afterwards were released, but none laid eggs. A second attempt to obtain eggs was done with 22 females from the population at Ming Ming Swamp, which were fully mature judging by their enlarged abdomen. Females were maintained with humid filter paper from 12:15 to 15:30 h, but once more no oviposition was observed. Five females were retained and maintained overnight with humid filter paper and a piece of vegetation, but yet again no eggs were laid.

4 Conclusions

Hemiphlebia mirabilis is a remarkable damselfly, as previous workers have pointed out (Fraser 1955; Davies 1985; Trueman 1999; New & Whelan 2003; New 2007). The observations and analyses presented here (which have to be treated as preliminary) indicate that this species has a copulatory behaviour similar to other Zygoptera, and sperm removal is likely to be performed by males. The specimens that were collected and preserved in ethanol, will be dissected in the next months, and hopefully a detailed description of sperm competition will be possible.

H. mirabilis males perform an elaborated courtship display, which uses in part its well-known flicking behaviour. This was an unexpected result, and suggests that precopulatory sexual selection might be intense in this species.

I was able to observe and describe reproductive behaviour (goal 1 of this work), but I could not observe oviposition, neither elicit egg-laying on humid filter paper and plant tissue. Therefore, the second goal of the study, to develop methods for captive breeding, could not be achieved. Nevertheless, I dissected eggs from two mature females and preserved them in a buffer for transmission electron microscopy. Their study will be done during 2014.

The dense vegetation of Long Swamp was too thick for behavioural observations. If females lay eggs at the base of the reeds, this is unlikely to be observed. Even mating pairs were very difficult to detect among the vegetation. Furthermore, individuals in copula were never seen flying, which also difficults detection. The fact that no focal female attempted oviposition suggests that this behaviour might take place during the night. Nevertheless, five females were maintained over night with humid filter paper, and did not lay eggs. The structure of the vegetation at Ming Ming Swamp (Figure 30) is more favourable for behavioural observations. *H. mirabilis* was very common when I visited the swamp, and I think that detailed observations at that place would be fruitful to detect oviposition.

My mark-recapture experiment, with all the limitations inherent to a study made by only one worker, yielded surprisingly high population density estimates, which are concordant with field observations. Davies (1985) estimated a density of 100 animals per 10 m^2, which is three times my estimates, but at particular times I observed similar densities. The population of *H. mirabilis* at Long Swamp is therefore huge, very likely more than one million specimens per season. Its has also been found in large numbers in several swamps in Grampians National Park (Reiner Ritcher, pers. comm). I therefore agree with the opinion of other researchers, which suggested that this species should no longer be regarded as critically endangered (Trueman et al. 1992; Watson 1995).

Figure 30. A view of Ming Ming Swamp, Grampians National Park.

5 Acknowledgements

This work could not be possible without the help of many colleagues, which shared with me their experiences and information about the enigmatic *Hemiphlebia mirabilis* and other Australian species. Many thanks to Di Crowther, Ian Endersby, John Hawking, Reiner Ritchter, and Richard Rowe. Gerry Quinn and the staff at the Warrnambool campus of Deakin University helped to solve logistic problems. The staff at Victorian Department of Environment and Primary Industries

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7 Annex. List of specimens captured.

Coordinates are in WGS84 Datum. Some specimens could not be identified to species yet.

Specimen code	Genus	species	sex	Family	Date	place capture	locality	X-coordinate	Y-coordinate	altitude (m)
ACR-00715	*Hemiphlebia*	*mirabilis*	male	Hemiphlebiidae	18/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00716	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	18/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00717	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	25/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00718	*Hemiphlebia*	*mirabilis*	male	Hemiphlebiidae	26/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00719	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	26/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00720	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	26/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00721	*Hemiphlebia*	*mirabilis*	male	Hemiphlebiidae	26/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00722	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	26/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00723	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	26/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00724	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	27/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00725	*Austrolestes*		female	Lestidae	27/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00726	*Austrolestes*		female	Lestidae	27/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00727	*Austrolestes*		male	Lestidae	27/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00728	*Ischnura*	*heterosticta*	male	Coenagrionidae	27/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00729	*Ischnura*	*heterosticta*	male	Coenagrionidae	27/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00730	*Ischnura*	*heterosticta*	male	Coenagrionidae	27/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00731	*Austrolestes*		female	Lestidae	28/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00732			female	Libellulidae	27/11/2013	Long Swamp	Nelson	141.13672	-38.10997	5
ACR-00733	*Hemicordulia*	*?tau*	male	Hemicorduliidae	25/11/2013	Forest road	Nelson	141.15142	-38.083981	30
ACR-00734	*Ischnura*	*heterosticta*	female	Coenagrionidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00735	*Ischnura*	*heterosticta*	male	Coenagrionidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00736	*Austrolestes*		female	Lestidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00737	*Austrolestes*		male	Lestidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11

Specimen code	Genus	species	sex	Family	Date	place capture	locality	X-coordinate	Y-coordinate	altitude (m)
ACR-00738	*Ischnura*	*aurora*	male	Coenagrionidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00739	*Ischnura*	*heterosticta*	male	Coenagrionidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00740			male	Aeshnidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00741	*Austrolestes*		male	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00742	*Austrolestes*		male	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00743	*Austrolestes*		male	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00744	*Austrolestes*		male	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00745	*Austrolestes*		male	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00746	*Austrolestes*		female	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00747	*Austrolestes*		female	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00748	*Austrolestes*		female	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00749	*Austrolestes*		female	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00750	*Austrolestes*		female	Lestidae	01/12/2013	Bushland Retreat	Portland	141.4821	-38.334663	69
ACR-00751	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	29/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00752	*Hemiphlebia*	*mirabilis*	male	Hemiphlebiidae	29/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00753	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	29/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00754	*Hemiphlebia*	*mirabilis*	male	Hemiphlebiidae	29/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00755	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	29/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00756	*Ischnura*	*heterosticta*	male	Coenagrionidae	29/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00757	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	30/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00758	*Hemiphlebia*	*mirabilis*	copula	Hemiphlebiidae	30/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00759	*Hemiphlebia*	*mirabilis*	male	Hemiphlebiidae	30/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00760	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	30/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00761	*Hemiphlebia*	*mirabilis*	male	Hemiphlebiidae	30/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00762	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	30/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11

Specimen code	Genus	species	sex	Family	Date	place capture	locality	X-coordinate	Y-coordinate	altitude (m)
ACR-00763	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	30/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00764	*Hemiphlebia*	*mirabilis*	copula	Hemiphlebiidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00765	*Hemiphlebia*	*mirabilis*	copula	Hemiphlebiidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00766	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00767	*Hemiphlebia*	*mirabilis*	male	Hemiphlebiidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00768	*Hemiphlebia*	*mirabilis*	copula	Hemiphlebiidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00769	*Hemiphlebia*	*mirabilis*	copula	Hemiphlebiidae	01/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00770	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	29/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00771	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	29/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00772	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	29/11/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00773	*Austrolestes*	*leda*	male	Lestidae	02/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00774	*Austrolestes*	*leda*	male	Lestidae	02/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00775	*Austrolestes*	*leda*	male	Lestidae	02/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00776	*Ischnura*	*aurora*	female	Coenagrionidae	02/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00777	*Austrolestes*	*leda*	female	Lestidae	02/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00778	*Ischnura*	*heterosticta*	female	Coenagrionidae	03/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00779	*Austrolestes*		female	Lestidae	03/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00780	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	03/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00781	*Hemiphlebia*	*mirabilis*	copula	Hemiphlebiidae	03/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00782	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	03/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00783	*Hemiphlebia*	*mirabilis*	copula	Hemiphlebiidae	03/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00784	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	03/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00785	*Hemiphlebia*	*mirabilis*	copula	Hemiphlebiidae	03/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00786	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	06/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00787	*Austrolestes*	*leda*	male	Lestidae	04/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00788	*Austrolestes*	*leda*	male	Lestidae	04/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215

Specimen code	Genus	species	sex	Family	Date	place capture	locality	X-coordinate	Y-coordinate	altitude (m)
ACR-00789	*Austrolestes*	*leda*	female	Lestidae	04/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00790	*Austrolestes*	*leda*	female	Lestidae	04/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00791	*Austrolestes*	*leda*	female	Lestidae	04/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00792	*Austrolestes*	*leda*	female	Lestidae	04/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00793	*Ischnura*	*aurora*	female	Coenagrionidae	04/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00794	*Ischnura*	*aurora*	female	Coenagrionidae	04/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00795	*Ischnura*	*aurora*	female	Coenagrionidae	04/12/2013	Ming Ming Swamp	Grampians National Park	142.20991	-37.321075	215
ACR-00796			male	Coenagrionidae	07/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00797	*Hemiphlebia*	*mirabilis*	female	Hemiphlebiidae	06/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00798	*Hemiphlebia*	*mirabilis*	6 males	Hemiphlebiidae	07/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00799	*Hemiphlebia*	*mirabilis*	9 males	Hemiphlebiidae	07/12/2013	Long Swamp	Nelson	141.13747	-38.110307	11
ACR-00800	*Ischnura*	*heterosticta*	male	Coenagrionidae	09/12/2013	West Kiewa river	Mount Beauty	147.16303	-36.739814	347
ACR-00801	*Ischnura*	*heterosticta*	female	Coenagrionidae	09/12/2013	West Kiewa river	Mount Beauty	147.16303	-36.739814	347
ACR-00802	*Ischnura*	*heterosticta*	female	Coenagrionidae	09/12/2013	West Kiewa river	Mount Beauty	147.16303	-36.739814	347
ACR-00803	*Ischnura*	*heterosticta*	female	Coenagrionidae	09/12/2013	West Kiewa river	Mount Beauty	147.16303	-36.739814	347
ACR-00804	*Ischnura*	*heterosticta*	female	Coenagrionidae	09/12/2013	West Kiewa river	Mount Beauty	147.16303	-36.739814	347
ACR-00805	*Xanthagrion*	*erythroneurum*	male	Coenagrionidae	09/12/2013	West Kiewa river	Mount Beauty	147.16303	-36.739814	347
ACR-00806	*Xanthagrion*	*erythroneurum*	male	Coenagrionidae	09/12/2013	West Kiewa river	Mount Beauty	147.16303	-36.739814	347
ACR-00807	*Austroargiolestes*	*icteromelas*	male	Megapodagrionidae	09/12/2013	West Kiewa river	Mount Beauty	147.16303	-36.739814	347
ACR-00808	*Austroargiolestes*	*icteromelas*	male	Megapodagrionidae	09/12/2013	Kiewa river	Coral Bank	147.09314	-36.58377	266
ACR-00809	*Austroargiolestes*	*icteromelas*	male	Megapodagrionidae	09/12/2013	Kiewa river	Coral Bank	147.09314	-36.58377	266
ACR-00810	*Austroargiolestes*	*icteromelas*	male	Megapodagrionidae	09/12/2013	Kiewa river	Coral Bank	147.09314	-36.58377	266
ACR-00811	*Austroargiolestes*	*icteromelas*	female	Megapodagrionidae	09/12/2013	Kiewa river	Coral Bank	147.09314	-36.58377	266
ACR-00812	*Ischnura*	*heterosticta*	female	Coenagrionidae	09/12/2013	Kiewa river	Coral Bank	147.09314	-36.58377	266
ACR-00813	*Austroagrion*	*watsoni*	male	Coenagrionidae	09/12/2013	Kiewa river	Coral Bank	147.09314	-36.58377	266
ACR-00814	*Austroargiolestes*	*icteromelas*	male	Megapodagrionidae	09/12/2013	Kiewa river	Kegünyah	147.03127	-36.328149	191

Specimen code	Genus	species	sex	Family	Date	place capture	locality	X-coordinate	Y-coordinate	altitude (m)
ACR-00815	*Austroargiolestes*	*icteromelas*	female	Megapodagrionidae	09/12/2013	Kiewa river	Kegünyah	147.03127	-36.328149	191
ACR-00816	*Austroargiolestes*	*icteromelas*	female	Megapodagrionidae	09/12/2013	Kiewa river	Kegünyah	147.03127	-36.328149	191
ACR-00817	*Austroargiolestes*	*icteromelas*	female	Megapodagrionidae	09/12/2013	Kiewa river	Kegünyah	147.03127	-36.328149	191
ACR-00818	*Ischnura*	*aurora*	male	Coenagrionidae	09/12/2013	pond at Bandiana	Wodonga	146.91918	-36.144336	180
ACR-00819	*Ischnura*	*aurora*	male	Coenagrionidae	09/12/2013	pond at Bandiana	Wodonga	146.91918	-36.144336	180
ACR-00820			female	Coenagrionidae	09/12/2013	pond at Bandiana	Wodonga	146.91918	-36.144336	180
ACR-00821	*Nososticta*	*solida*	female	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00822	*Nososticta*	*solida*	male	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00823	*Nososticta*	*solida*	male	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00824	*Nososticta*	*solida*	female	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00825	*Nososticta*	*solida*	female	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00826	*Nososticta*	*solida*	female	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00827	*Nososticta*	*solida*	male	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00828	*Nososticta*	*solida*	male	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00829	*Nososticta*	*solida*	female	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00830	*Nososticta*	*solida*	male	Protoneuridae	09/12/2013	House creek	Wodonga	146.87947	-36.143721	160
ACR-00831	*Austroargiolestes*		female	Megapodagrionidae	10/12/2013	Snowy creek	Mitta Mitta	147.37775	-36.536272	270
ACR-00832	*Austroargiolestes*		male	Megapodagrionidae	10/12/2013	Snowy creek	Mitta Mitta	147.37775	-36.536272	270
ACR-00833	*Austroargiolestes*		male	Megapodagrionidae	10/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365
ACR-00834	*Austroargiolestes*		female	Megapodagrionidae	10/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365
ACR-00835	*Austroargiolestes*		female	Megapodagrionidae	10/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365
ACR-00836	*Synlestes*	*weyersii*	female	Synlestidae	10/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365
ACR-00837	*Diphlebia*	*lestoides*	male	Diphlebiidae	10/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365
ACR-00838	*Eusynthemis*	*brevistyla*	male	Synthemistidae	10/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365
ACR-00839	*Austroargiolestes*	*calcaris*	male	Megapodagrionidae	10/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365
ACR-00840	*Austroargiolestes*	*calcaris*	male	Megapodagrionidae	10/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365

Specimen code	Genus	species	sex	Family	Date	place capture	locality	X-coordinate	Y-coordinate	altitude (m)
ACR-00841	*Diphlebia*	*lestoides*	male	Diphlebiidae	11/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365
ACR-00842	*Diphlebia*	*lestoides*	male	Diphlebiidae	11/12/2013	Snowy creek	Walnuts	147.42638	-36.614308	365
ACR-00843	*Austroargiolestes*	*calcaris*	female	Megapodagrionidae	11/12/2013	small tributary of Snowy creek	Harker creek	147.42323	-36.620893	394
ACR-00844	*Austroargiolestes*	*calcaris*	female	Megapodagrionidae	11/12/2013	small tributary of Snowy creek	Harker creek	147.42323	-36.620893	394
ACR-00845	*Eusynthemis*	*brevistyla*	male	Synthemistidae	11/12/2013	small tributary of Snowy creek	Harker creek	147.42323	-36.620893	394
ACR-00846	*Eusynthemis*		male	Synthemistidae	12/12/2013	Seven Creeks at Gooram falls	Gooram	145.63313	-36.903484	235
ACR-00847	*Austrogomphus*		male	Gomphidae	12/12/2013	Seven Creeks at Gooram falls	Gooram	145.63313	-36.903484	235
ACR-00848	*Austrogomphus*		female	Gomphidae	12/12/2013	Seven Creeks at Gooram falls	Gooram	145.63313	-36.903484	235
ACR-00849	*Austrogomphus*		female	Gomphidae	12/12/2013	Seven Creeks at Gooram falls	Gooram	145.63313	-36.903484	235
ACR-00850	*Ischnura*	*aurora*	female	Coenagrionidae	09/12/2013	Kiewa river	Coral Bank	147.09314	-36.58377	266