

## Observations on rearing damselflies under laboratory conditions

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**Abstract**—Rearing damselflies under laboratory conditions is a promising means of solving a variety of biological questions. Therefore, in order to improve the success of future researchers we felt the need to indicate potential difficulties in carrying out rearing experiments. Laboratory crosses were obtained using virgin animals originating from natural populations in Belgium and Spain. Resulting offspring was maintained, under laboratory conditions, in small aquaria until emergence and in insectaries as adults. Our results show that keeping damselflies during their entire life cycle under artificial conditions can be very difficult. We suggest that future researchers should change water regularly, supply sufficient food, and rear animals at low density or even individually. Furthermore, suggestions are given on type of food, advisable laboratory conditions and female oviposition methodology.

*Keywords:* damselflies; experimental set up; laboratory crosses; rearing.

### INTRODUCTION

Over the last few decades, damselflies have become popular study organisms to examine a variety of biological topics (Fincke et al., 1997). One of their merits compared to vertebrates is a huge diversity in reproductive strategies and the convenience of being able to collect lifetime data over a short period of time. Although most studies on damselflies were performed in the field (Corbet, 1999), possibilities exist to study captive imagines in outdoor insectaries where it is possible to reduce the uncontrolled conditions inherent to conventional field

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studies (Van Gossum et al., 1999). Rearing larval damselflies also turned out to be fairly easy (Krull, 1929; Sweetman and Laudani, 1942; Johnson, 1991). Maintaining damselflies under laboratory conditions during several generations, however, appears to cause problems (e.g., Cordero, 1990; Andrés and Cordero, 1999; Crudgington, Sirot, pers. comm.; but see Whedon, 1942). Nevertheless, rearing experiments serve to answer many questions, hence success in carrying them out is important (Whedon, 1942). Indeed, rearing experiments have great potential for answering evolutionary and ecological questions. For example, assessment of offspring allows us to study genetic and environmental variability in traits (Maynard Smith, 1989). Furthermore, the possibility is offered to verify if lifetime mating success is a good predictor of lifetime reproductive success, which is commonly assumed in most lifetime studies (e.g., Harvey and Walsh, 1993; Richardson and Baker, 1997; Stoks, 2000; but see Fincke and Hadryś, 2001). The aim of this paper is to comment on observations on the rearing of damselflies under laboratory conditions. This should enable future researchers to avoid the problems we suffered in the rearing of larvae and adults.

## MATERIAL AND METHODS

We performed a rearing experiment at two locations, Belgium and Spain, using slightly different methodology as explained below. In each paragraph we will explain how the experiments were performed in Belgium and Spain, respectively.

Adults of the study species, *Ischnura elegans*, were collected from natural populations in Niel, Northern Belgium, at the beginning of June 1999, and at Louro coastal lagoon, Muros, A Coruña province, Galicia, NW Spain, during September 1999 and June 2000. Captured animals were 0-1 day old, and were exhibiting a dull body coloration and a vitreous shine in the wings (Miller, 1987). These individuals were sexually immature and consequently virgin. Animals were placed in empty film tubes (Belgium) or small net containers (Spain) which were kept in the shade, and transported to the laboratory as soon as possible (less than 2 hours after capture). Upon capture every individual received a unique number on its wings written in black ink with a permanent marker (Staedtler Pancolor®, 0.3 mm pen). Handling and marking may affect survival probabilities (Cordero et al., 2001), but is essential to recognise individuals. Animals in Belgium were maintained upon maturation in insectaries (50 × 50 × 70 cm wooden construction covered with bee-netting 0.5 × 0.5 mm, 15-20 animals per insectary) under laboratory conditions (20°C, 80-90% humidity and a photo-period of 16L : 8D hours, see also Cordero, 1990). In the insectaries dried *Juncus effusus* was provided as perching substrate. Commercially available plastic culture boxes of *Drosophila* sp. were obtained as food resource for the damselflies. To prevent damselflies from getting stuck in the medium of the *Drosophila* culture, the plastic boxes were covered. Holes, sufficiently big to allow free movement of *Drosophila*, but which prevented the entry of damselflies (see also Cooper et al., 1996) were punched in the cover. Additionally, twice a day all

insectaries underwent a shower to sustain humid conditions and to avoid the drying out of animals (Corbet, 1999). In the insectaries in Spain (50 × 50 × 50 cm, wooden construction with 2 mm glass, 15-20 animals per insectary), wooden sticks were provided as perches while the internal part of the cages was lined with aluminium foil, which reflects the light and minimises escape behaviour (Cooper et al., 1996; Andrés and Cordero, 1999). Insectaries were maintained at room temperature, and indirectly (to protect animals from direct sunlight) received solar illumination through windows. During overcast days, artificial light was provided by situating a lamp (60 watts) over each insectary (10 cm). A data logger (Gemini Data Loggers, Chichester, UK) was introduced to register temperature inside one of the insectaries, which was considered a model for the temperature variation in the other insectaries. Cultures of *D. melanogaster* (Meigen, 1830) were maintained as a food resource for the damselflies. The flies were reared using standard procedures, with a diet based on corn, water, agar, salt, yeast, ethanol (96°) and Nipagin. A covered water supply, to prevent animals from drowning, was provided in each insectary to sustain humid conditions. In both Belgium and Spain, males and females were maintained separately in insectaries.

Insectaries to maintain imagines (see previous paragraph) were also used to obtain laboratory matings and to keep adult animals. Single receptive females were introduced into an insectary containing several sexually mature males (5-15 males) (both male and female 5-8 days old, see Cordero et al., 1998 for maturation time in *I. elegans*). After mating, the female was isolated (to avoid additional copulations) in an insectary (identical to the ones to maintain imagines) with abundant *Drosophila* to enable the female to compensate her energy expenditure for copulation. In Belgium in each insectary a water-filled (5 mm) Petri dish with embedded filter paper was provided. The paper was labelled with the identity of the parents (the numbers of male and female previously in copulation) and the date. Filter papers were removed daily from the cages during the morning and maintained in small aquaria filled with pond water. Only those papers containing eggs (which could be detected by holding the papers under a light source) were retained. In Spain a slightly different approach was used. After mating, females were introduced singly into small vials containing only wet filter paper, which is sufficient to elicit oviposition in the study species (Cordero, 1990). Females were kept solitary in the vial for several hours to obtain clutches. Afterwards, they were placed again into the insectaries, together with other females, and abundant *Drosophila*. This process was repeated during subsequent days in order to maximise the number of clutches.

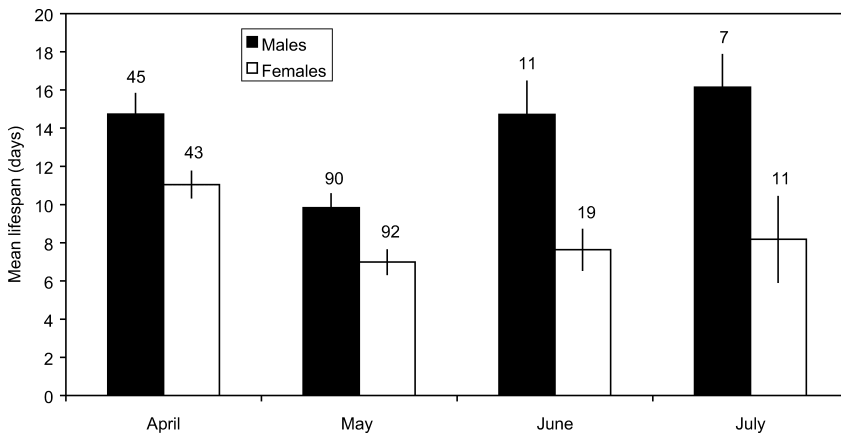
Eggs were maintained in the filter paper in small aquaria (for each female a different aquarium was used) filled with water. Larvae emerged after 2 weeks to 1 month and were maintained in the same aquaria (up to 50 larvae per aquarium) with strips of filter paper supplied as perches. Throughout the larval stage, animals were fed daily with nauplii of *Artemia salina* (Cordero, 1990). Upon reaching a size of 5 mm, they received additional food, i.e. *Lumbriculus variegatus* (Müller, 1774). In Belgium, pond water in the aquarium was not refreshed, but aquaria were

oxygenated using air pumps (Sweetman and Laudani, 1942). After 2 months, water was changed because of an increasing abundance of algae and a high degree of mortality of larvae. In Spain, larvae were maintained in small aquaria filled with tap (pH 9; first generation) and spring water (pH 7; second generation). Tap water was purified using active-carbon filters, to avoid problems associated with chloride. Water was not oxygenated but was changed every 2-7 days, depending on pollution of the aquaria, to avoid anoxia. Furthermore, dead individuals were removed each time water was changed in order to avoid the growth of fungi in the cultures (in our experience fungi develop on dead larvae after 1 or 2 days). Mortality was recorded every time the water was changed once larvae reached ca. 5 mm. Larvae reaching their final stage were isolated and a wooden stick was provided as emergence substrate. Emerged individuals were raised in the same type of insectaries as the parental generation until mature coloration. Some individuals were used for further cross experiments to obtain second-generation progenies.

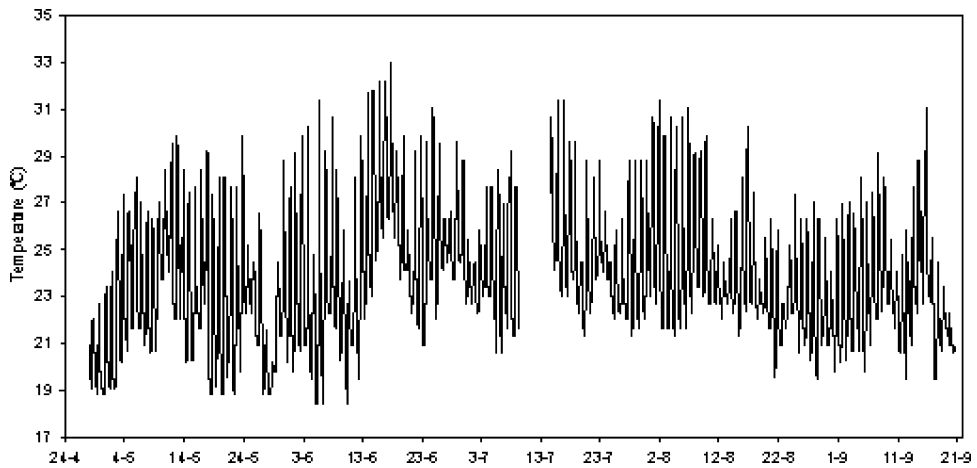
## RESULTS

The first attempts in Belgium to maintain virgin animals in the insectaries upon maturation failed because of insufficient *Drosophila* to prey on (less than five flies per day per damselfly). If abundant prey was provided (some 100 flies per day per insectary of ten damselflies; Hinnekint, 1987) most damselflies survived the maturation time. A second problem was encountered with inducing females to deposit their eggs. It appeared that females suffered difficulties finding the water in the insectaries and, if they did encounter it, sometimes drowned. Nevertheless the first generation progeny in Belgium consisted of 18 crosses of which approximately 1200 larvae were maintained. After 2 months, unfortunately, larvae appeared to die off fast (30-40 animals each day). Although oxygen pumps were used, mortality could possibly be explained by the increasing quantity of algae in the water. However, it is more likely that insufficient food was the reason for the observed mortality. Although water was changed and more food was given (twice a day instead of once) the mortality continued. After 2 weeks most larvae were dead and none of the performed crosses resulted in emerging damselflies.

For the first generation in Spain 12 crosses were obtained where larvae emerged from the eggs. Because mortality rates turned out to be high, it was decided to change from tap to spring water (30 December 1999). Spring water came from a source of drinking water and therefore is unlikely to contain high amounts of micro-organisms. Surviving and emerging animals suggested that spring water was preferable. The development of the larvae was slower during the first generation (5-8 months before emergence) than during the second generation (2-5 months). Twelve second-generation crosses were obtained. Unfortunately, on the moment of emergence the number of imagines appeared to be fairly low. It is probable that insufficient food during the first months of larval life caused a high degree of cannibalism among the larvae, hence reducing the number of emerging animals.



**Figure 1.** Average lifespan of adult *Ischnura elegans* that emerged in the laboratory, as a function of their date of emergence. Numbers inside bars indicate sample size.



**Figure 2.** Temperature variation inside the insectaries from April to September 2000.

In April-June 1999 (first generation) and July 2000 (second generation) we measured imagine life span of 153 males and 164 females from the experiment in Spain. A two-way ANOVA indicated that sex ( $F_{1,316} = 27.86$ ,  $p < 0.0001$ ) and month of emergence ( $F_{3,316} = 10.65$ ,  $p < 0.0001$ ) had a significant effect on life span (fig. 1). Males lived 15-16 days, but adult females lived on average only 7-11 days. Figure 2 shows the temperature measured inside the insectaries during the period of study. In late May there were several days with low temperatures, due to rainy weather. During these episodes mortality increased.

## DISCUSSION

As should be clear from our results, maintaining life cycles of damselflies under laboratory conditions demands a lot of labour and many things can cause the experiment to fail (Cordero, 1990; Andrés and Cordero, 1999). We will discuss potential problems and make the following suggestions on maintaining both imagines and larvae.

- 1) To minimise the risk to damage body structures, we suggest refraining from writing numbers on animals which have just emerged, This could easily be accomplished by maintaining the animals isolated for 24 h before marking.
- 2) Feeding the imagines is complicated because *Drosophila* flies concentrate in the corners of the insectaries where they are protected from damselfly predation (this can be prevented by using circular insectaries (a comment made by one of the referees)). Even in the presence of several hundred flies in the insectaries some damselflies may suffer food limitation. Therefore it is important to disperse flies over the insectaries at least once a day, but better results are obtained by making flies fly around three or four times per day.
- 3) Inducing females to deposit eggs in insectaries with a water supply (as in the experiment in Belgium) caused problems because several females could not find the water or even drowned. Inducing oviposition was easy if females were kept in small vials containing only wet filter paper as a perch (as in the experiment in Spain). Furthermore, introducing the abdomen of the female into water seems to elicit female oviposition behaviour. Great care should be taken with ovipositing females, because the damp filter paper sometimes acts as a trap and wings are easily damaged. After oviposition, it is useful to feed the females by hand by offering dead *Drosophila* with forceps (see Hinnekint, 1987). We recommend supplying *Drosophila* only after oviposition when the females are returned to the insectaries.
- 4) It was found that adult males survived better than females, which is in contrast with previous results with four Coenagrionid damselflies, including *Ischnura graellsii* and *I. pumilio* (Cordero, 1994; but see Rowe, 1987). A higher food requirement by females (ovarian maturation) compared with males during the post-emergence period might explain this gender effect (Anholt et al., 1991; Falck and Johansson, 2000).
- 5) Seasonal differences, probably temperature and photoperiod, affected the life span of the animals.
- 6) The lower life span during May was probably due to overcrowding, because most adults of the first generation emerged at that time.

For maintenance of larvae several things should be taken into account. Firstly, a probable contributing factor for the failure of the Belgium experiment was insufficient changing of the aquaria water. Moreover, starvation of animals was due to an insufficient supply of food which resulted in a rapid mortality of the animals

under study. When the conditions for the larvae were improved it was probably too late, and therefore the animals, which were living under stress, could not be saved. Secondly, the first generation in the experiment in Spain suffered a high mortality during the first weeks and a slow development during the whole experiment. The major cause of mortality was probably the use of tap water (identical observations, unpublished results J. Andrés), and winter temperature and light conditions slowed down the rearing process (De Block, unpubl.). However, in a current breeding experiment with *Ischnura hastata*, mortality remained constant (and low), although we changed from spring to tap water. Avoiding the need to collect spring water every week saves a lot of time. Most probably some kind of toxic substance is released from tap water pipes in new buildings, and this disappears after a couple of years (Svensson, pers. comm.). Because the building in Spain was less than 1 year old when *I. elegans* breeding started, this is a probable reason for problems with tap water and mortality in larvae.

Thirdly, rearing the second generation of progeny in Spain failed due to insufficient food during the first months of larval life, causing a high degree of cannibalism among the larvae (e.g., Johnson, 1991; but see Thompson, 1978). To avoid this problem, the only possibility is to dedicate many hours to maintenance of culture. To conclude, it is advisable to maintain larvae individually to avoid cannibalism. This method, however, is very time-consuming because of the need to change water and to add food on a regular basis (Cordero, 1990). Much time can be gained by placing larvae in individual cells but sharing the same water supply: this reduces the frequency of water changing and standardises breeding conditions. Another possibility, which has worked satisfactorily with *Ceriatrion tenellum*, is to maintain larvae at a very low density (Andrés and Cordero, 1999).

We hope that future investigators, applying our suggestions, will be more successful in performing crosses and maintaining damselflies over several generations under laboratory conditions.

### *Acknowledgements*

We are very grateful to Marjan De Block, Luc De Bruyn, Jan Scheirs and Robby Stoks for their comments and criticisms on previous versions of the manuscript. We would also like to thank Kris Lauwers and Wim Van Gossum for their assistance during the construction of the insectaries for the experiment in Belgium, and Luc De Bruyn and Rob Nelissen for sharing the frustrations of trying to obtain crosses and rearing larvae in Antwerp. HVG was supported by an IWT-grant and a travel-residence grant from the Fund for Scientific Research Flanders (FWO). This work was also partly supported by research funding to ACR from the Spanish Ministry of Science and Culture (Dirección General de Enseñanza Superior, project code PB97-0379).

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