

## THE FUNCTIONS OF WINGCLAPPING IN THE CALOPTERYGIDAE (ZYGOPTERA)

P.L. MILLER

Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, United Kingdom

*Received September 5, 1993 / Reviewed and Accepted October 4, 1993*

The occurrence and function of wingclapping have been examined in the field and in the laboratory in *Calopteryx splendens*, *C. xanthostoma*, *C. virgo* and *C. haemorrhoidalis*. Wingclapping, which occurs commonly after flight, involves a slow wing depression by up to 160° (mean duration 735 ms; maximum, 1300 ms), followed by a rapid elevation (mean duration 157 ms; maximum, 200 ms), with a mean ratio of 4.68:1.0. In the field, wingclaps were observed after 33.6% of all flights in *C. splendens*, at 24-25°C (ambient shade temperature), 5 times more commonly in males than in females; they occurred after 32% of all flights in *C. xanthostoma* at 30°C and after 80% of all flights in *C. haemorrhoidalis* at 30°C, with a similar proportion in each sex. — In the laboratory at 30°C wingclapping commonly followed tethered flights, and sometimes also walking and grooming. The number of wingclaps was not correlated with the duration of the preceding flight either in the field or in the laboratory. Spontaneous claps (i.e. those not following other activity) became commoner at 33-34°C, and also in hypoxic atmospheres. Additionally teneral adults, experiencing oxygen shortage, commonly made 3 types of movement not seen in more mature adults, but resembling larval respiratory responses. These were side-to-side shaking movements (often accompanied by a single wingclap), sustained wing opening by 20-40° and periodic body raising ('press-ups'). — Measurements have shown that in *C. splendens* up to 1.4 µl air stroke-1 is displaced from the thorax by forced wing movements, indicating that about 14 µl g-1 stroke-1 could be autoventilated during flight, a figure comparable to T. WEIS-FOGH's (1967, *J. exp. Biol.* 47: 561-587) values for aeshnids. It is concluded that wingclapping may serve both respiratory and thermoregulatory functions, the latter by circulating air internally and externally. Wingclapping may be necessary in calopterygids because of their relatively large size and inefficient abdominal ventilatory pump.

### INTRODUCTION

Many calopterygid damselflies possess prominently marked wings. A variety of types of movement of the wings, both during flight and when perched, has

been described and some have been interpreted as signals, for example in connection with territorial tenancy, male-male aggression or courtship (BICK & BICK, 1965, 1978; HEYMER, 1973; WAAGE, 1984, 1988; RÜPPELL, 1985, 1989). One type of wing movement, commonly observed in perched calopterygids after a flight, consists of a slow wing depression followed by a rapid elevation back to the resting position with the wings more or less in line with the abdomen. ERICKSON & REID (1990) have pointed out that this movement, termed wing-clapping, occurs in many non-social contexts, and they have provided evidence, based on observations of the rate of clapping at different temperatures and on the number of claps which follow feeding flights, that it has a thermoregulatory role. They suggest that the wing movements cool the thorax by blowing air over it externally. However wing movements are also likely to ventilate the thoracic tracheal system and they may thus cool the thorax internally as well as supplying it with oxygen. Indeed respiratory and thermoregulatory roles may be difficult to distinguish from one another.

Observations on the occurrence of wingclapping at different temperatures in the field and laboratory are reported here. WEIS-FOGH (1967) made measurements of the thoracic volume changes caused by wing movements in aeshnid dragonflies and he concluded that such movements made a major contribution to respiration during flight. By using a similar technique, the volume of air displaced by forced wing movements has been measured in *C. splendens* and it has led to the hypothesis that wingclapping provides an important respiratory as well as thermoregulatory mechanism in active damselflies.

## METHODS

*Calopteryx splendens* (Harris) were observed at streams in the vicinity of Oxford, U.K., in 1992 and 1993 (June-August), and *C. xanthostoma* (Charpentier), *C. virgo* (L.) and *C. haemorrhoidalis* (Vander Linden) in southern France on the Canal de Vergières (La Crau, Gard), and on the rivers Vis and Vidourle (Hérault), during July, 1993. Laboratory tests have been made only on *C. splendens*, either within a few hours of capture or after being kept overnight at 4°C. Teneral insects (within 24 h of emergence) were identified from their weak flight and shiny wings, and immature insects (post-teneral) were distinguished from matures by their colour and softer cuticle.

Ambient shade temperatures ( $T_a$ ) were recorded, but no attempt has been made to measure internal thoracic temperatures ( $T_{th}$ ). Flight durations were recorded together with the number of wingclaps after settling, using a hand-held voice recorder, the perch positions being noted with respect to exposure to the breeze and sun. Most flights recorded were of only a few seconds' duration, being mainly for feeding or patrolling. Longer flights involving interactions with conspecifics (male-male interactions, courtship) were treated separately. Values are given as means with their standard errors, and they were gathered from over two hundred individuals.

All behavioural observations in the laboratory were carried out on single insects so that interactions between individuals could not affect the observed behaviour. Individual *C. splendens* were 'tethered' by gluing a pin (38 x 0.55 mm) to one side of the thorax with "superglue" in such a way as not to interfere with wing or leg movements. Short bursts of flight were evoked by lifting the damselfly so that it faced into a light wind from a fan. Flight terminated when the legs were brought into

contact with the ground, and the pin was then immediately released. Experiments were carried out in a small room whose temperature was thermostatically controlled. Wingclapping was recorded on videotape for subsequent analysis.

To test the effect of oxygen shortage, damselflies were placed singly in transparent plastic boxes (172 x 115 x 50 mm) through which air and nitrogen mixtures, or air and carbon dioxide, were passed as previously described (MILLER, 1992). In some tests, 100% nitrogen was passed at 100-200 ml min<sup>-1</sup> into the observation box so that the insect experienced gradually increasing oxygen starvation. The temperature, measured in the box close to the insect, was controlled by submerging the box in a bath of heated or ice-cooled water.

Measurements of the thoracic volume changes which accompanied forced wing movements were made from a fresh, isolated thorax of *C. splendens* with the head, legs and abdomen removed, the wounds being sealed with a heated wax-resin mixture. All thoracic spiracles were sealed except for one spiracle 2 (on the meso-metathoracic boundary). A wax-resin dome was then built up round this spiracle and a tapered pipette sealed in with the tip close to the open spiracle valve (Fig. 1). The pipette led to a 1.5 mm diameter capillary tube in which a drop of eosin-alcohol acted as a marker.

The wings were moved through a fixed angle by gluing a thread to each and moving it along a scale. The excursion of the marker in the tube was measured under a microscope with an eye-piece graticule, which was then calibrated with a 10  $\mu$ l graduated pipette. That the pipette made an airtight fit with the tracheal system was tested by tilting the tube: if the marker moved, a leak was indicated and the preparation was re-sealed. Leaks could be located by gently injecting air into the thorax through the tube with the preparation under water. Air injection caused the wings to be depressed, while sucking out air caused wing elevation.

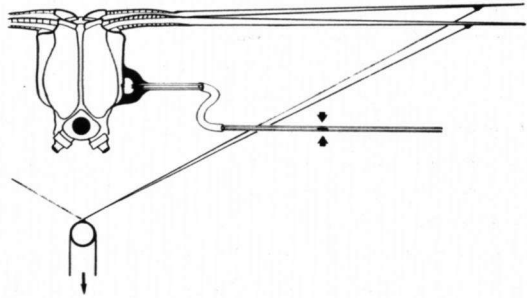


Fig. 1. Diagram to show the method of measuring volume changes in the thorax of *C. splendens* produced by wing movements. Wings are depressed by means of attached threads (single arrow). Volume changes are monitored by movements of a liquid marker in a tube (two arrows).

## FIELD OBSERVATIONS

In all the species observed, wingclaps occurred after a variable proportion of flights, more commonly at higher temperatures. The first wingclap usually appeared within a second of landing while others followed at increasing intervals (Fig. 2) and with a longer time course. The wings normally separated by 90-110° (maximally 160°), but miniature wingclaps with an amplitude of 20-40° also sometimes occurred. Wingclaps could readily be distinguished by their form from other types of wing movement such as the wing-spreading response to the approach of conspecifics (cf. HEYMER, 1973).

In *C. splendens* observed near Oxford at  $T_a$  24-25°C, 33.6% of all flights

(n=326) near a stream were followed by one or more wingclaps. Analysis of males and females separately showed that wingclaps occurred significantly more commonly in males than in females at this temperature [males: 79/187 (42%); females 7/84 (8.3%): chi squared,  $P < 0.001$ ]. In *C. haemorrhoidalis* at  $T_a$  29–31°C (10.00–12.00 h) on the Vergières canal, 80% (n=229) of flights were followed by one or more claps, whereas in *C. xanthostoma* at the same temperatures on the Vis, only 32% (n=78) were followed by claps. The frequency in *C. virgo* appeared to be similar to that of *C. xanthostoma*. There was no significant difference between the numbers of male and female *C. haemorrhoidalis* which performed wingclaps after flights [males, 79% (n=78); females 78% (n=35)].

Thus claps occurred about as commonly in *C. splendens* at  $T_a$  25°C as in *C. xanthostoma* at  $T_a$  30°C, whereas they were much commoner in *C. haemorrhoidalis* at the higher temperature. Wingclaps were commoner in male *C. splendens* than in females, but this was not the case in *C. haemorrhoidalis*.

At the high densities prevailing along the Canal de Vergières, *C. haemorrhoidalis* made many brief flights whose mean duration was  $3.8 \pm 0.94$  s (n=106;  $T_a$  30°C; 10.00–12.00 h). The number of wingclaps following flights was usually between 0 and 4 (maximally 12), the mean being  $2.31 \pm 0.21$  (n=106;  $T_a$  30°C). The number of wingclaps was not correlated with the duration of the preceding flight (Fig. 3): however after escalated highspeed interactions between males, which sometimes lasted for several minutes, there was no wingclap (n=7). ERICKSON & REID (1990) made a comparable observation on *C. maculata*.

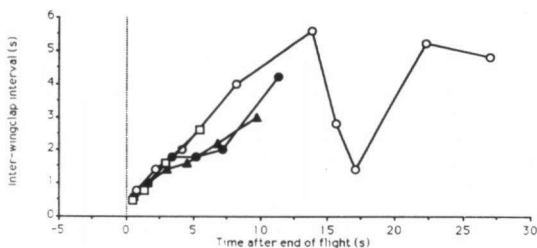


Fig. 2. Plot showing the increase in inter-wingclap intervals with time after four short flights in *C. splendens*. In one case 9 wingclaps followed the flight.

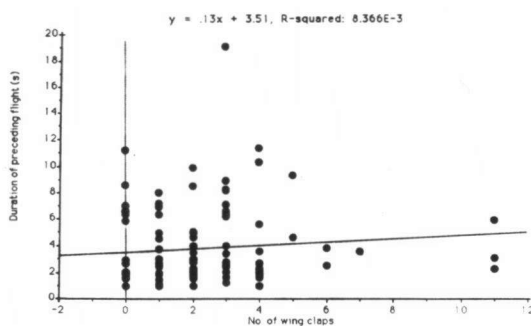


Fig. 3. Plot showing no significant regression of the duration of a tethered flight on the number of subsequent wingclaps in *C. haemorrhoidalis* recorded in the field at 30° C.

In order to compare *C. haemorrhoidalis* and *C. xanthostoma* more closely, observations were made along a bank of the river Vidourle where both species occurred in similar numbers and under the same environmental conditions ( $T_a$  29°C). Here, *C. haemorrhoidalis* made a mean of  $2.64 \pm 0.31$  post-flight wingclaps ( $n=45$ ), 9 flights (20%) being followed by no clap, whereas *C. xanthostoma* made a mean of  $1.01 \pm 0.255$  ( $n=52$ ), with 36 flights (69%) followed by no clap ( $P=0.0001$ ; 2-tailed t test). Thus it appears that wingclapping has a lower threshold in *C. haemorrhoidalis* than in *C. xanthostoma*.

### LABORATORY TESTS

Thoracic volume changes which accompany wing movements have been measured in seven *C. splendens* (Fig. 4). A maximum of  $1.4 \mu\text{l}$  air is sucked in when the wings are depressed through  $135^\circ$  and a similar volume is expelled when they are allowed to return elastically to the fully elevated position in line with the abdomen. The internal circulation of air which such changes produce could contribute both to the oxygen supply and to the cooling of the thorax provided that  $T_a$  was lower than  $T_{th}$ , as is likely to be the case in active damselflies (cf. MAY, 1991). However until internal thoracic temperatures are measured directly the contributions of wingclapping to thermoregulation will remain speculative.

The form and number of wingclaps observed in *C. splendens* after tethered flights or in plastic boxes in the laboratory were similar to those seen in the field. Wingclaps commonly occurred after flight, walking or grooming, and also sometimes spontaneously (i.e. not following any movement), particularly at high  $T_a$

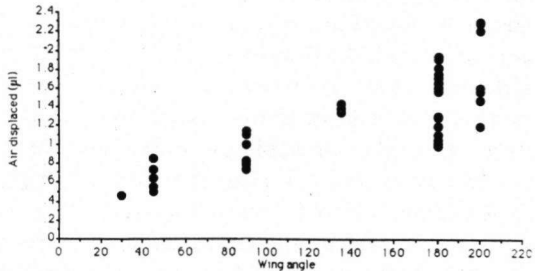


Fig. 4. Plot showing the relationship between the volume of air displaced and the wing angle in *C. splendens* ( $0^\circ$  = wings closed).

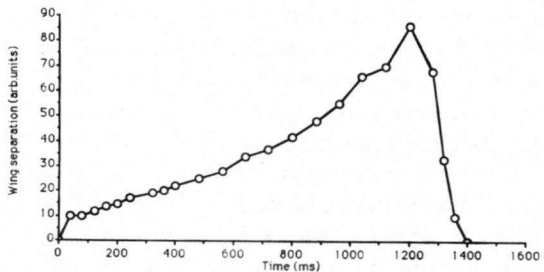


Fig. 5. Plot of the amount of wing separation (arbitrary units) against time (milliseconds) in a single wingclap, based on video recordings made in the laboratory on *C. splendens*.

(34°C). They took place only when the insect was making no other movement, except in teneral (see below).

Frame-by-frame analysis of video records shows that during a wingclap of about 135° amplitude, the wings open slowly with the hindwings slightly in advance, the movement taking up to 1300 ms (mean: 735 ms; range 320-1300; n=8); they are then closed rapidly (mean duration 157 ms; range 80-200; n=8), with the forewings well in advance of the hindwings (Fig. 5). The ratio of the opening to the closing duration has a mean of 4.68:1.0.

The number of wingclaps was not correlated with the duration of the preceding tethered flight (Fig. 6), in agreement with the field observations (Fig. 3). It was however found to be correlated with the prevailing temperature ( $P=0.0023$ ; Fig. 7). Since the metabolic rate is probably elevated at higher temperatures it was necessary to attempt to examine separately the responses of damselflies to hypoxia from their responses to temperature.

Most individuals, when subjected to hypoxic conditions at  $T_a$  25°C in plastic boxes, initially became more active, but with continued exposure they then became quiescent. Most forms of activity, including grooming, were commonly followed by one or more wingclaps, but spontaneous claps also increased in frequency in 23 out of the 45 insects tested. In one test carried out on 2 immature females, and 2 immature and 2 mature males, at  $T_a$  25.5°C, there was no spontaneous clap in air (5 min), but all six insects showed between 1 and 12 spontaneous claps when the box was perfused with nitrogen (5 min). Thus with hypoxia the frequency

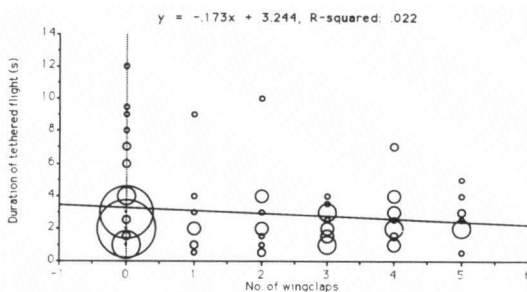


Fig. 6. Plot showing no significant regression of the number of wingclaps on the duration of the preceding tethered flight, measured in the laboratory in *C. splendens* ( $P=0.1234$ ).

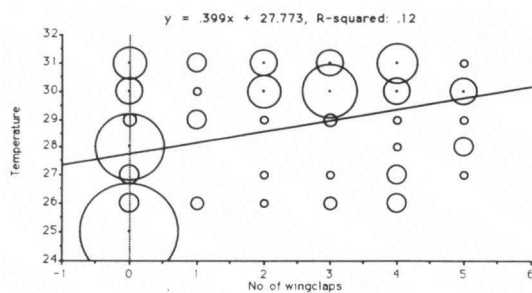


Fig. 7. Plot showing a significant regression of the number of wingclaps after a 2-5 s tethered flight on the ambient temperature. Data from 5 *C. splendens* ( $P=0.0003$ ).

of spontaneous claps was increased, at least in some individuals. That the response was not more strongly marked may be partly due to the fact that wingclapping in hypoxic atmospheres may actually increase the respiratory stress by sucking oxygen-deficient gases into the tracheal system.

Observations on 12 teneral *C. splendens* under hypoxic conditions revealed, in addition to an increase of wingclapping, three types of movement not seen in more mature individuals.

The first was a series of side-to-side shaking movements comparable to those performed by hypoxic larvae (Rütteln: ZAHNER, 1959; MILLER, 1993). Each bout of shaking was usually accompanied by a single wingclap, with the shaking being confined to the time when the wings were open (Fig. 8). Bouts of shaking in larvae are similar in duration to single wingclaps in adults. Secondly, with more pro-

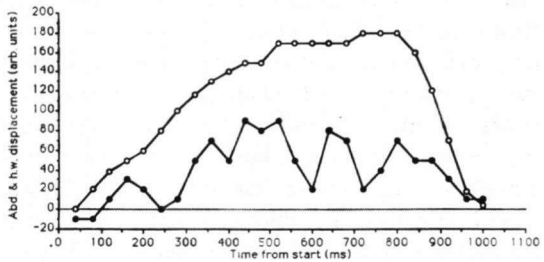


Fig. 8. Plot of hindwing opening (o----o) and lateral abdominal displacements (●-----●) (arbitrary units for both) against time (milliseconds) during a wingclap which is accompanied by lateral shaking movements, based on video recordings made from a teneral *C. splendens*.

longed hypoxia, the wings were held open by about 20–40°, a position comparable to that adopted by the wingbuds of late larvae when they experience oxygen shortage (ZAHNER, 1959; MILLER, 1993). Thirdly, teneral sometimes made regular body-raising movements by slight extensions of the legs ('press-ups'), a movement also observed in larvae (MILLER, 1993). Thus three types of activity which resemble larval respiratory responses can be evoked in adults but only in the teneral stage. Shaking is a well-recognised response of hypoxic larvae and the coupling of shaking to wingclaps in teneral suggests that wingclaps also are involved with respiration. As adult development progresses, shaking movements disappear but wingclaps continue to be expressed throughout adult life.

## DISCUSSION

Interpretations of the function of an activity usually depend on close observation of the activity under natural conditions, backed up by manipulation of the conditions in which it appears. Though wingclapping may sometimes function as a behavioural signal (BICK & BICK, 1978), the observations and experiments reported here suggest that more commonly it serves physiological roles, particularly at high temperatures when thermoregulation and an increased oxygen supply

may become necessary.

In mature adult *C. splendens* experimentally induced oxygen shortage produced only a small increase of wingclapping, whereas there was a more marked increase when  $T_a$  was raised, the latter in agreement with ERICKSON & REID (1990). Flight demands a high metabolic rate which produces much heat (MILL, 1985; CASEY, 1988); autoventilation by the wing movements supplies the extra oxygen while movement through the air provides convective cooling. At the end of flight, heat may briefly accumulate when movement through the air abruptly ceases. Likewise the oxygen deficit may momentarily increase if the high metabolic rate of flight declines more slowly than the sudden cessation of autoventilation. Wingclapping after flight may therefore provide relief from a temporary heat stress and a brief period of oxygen shortage. Other insects such as locusts, large flies, bees, wasps and dragonflies are known to continue abdominal pumping at a high rate for a few seconds after a flight (MILLER, 1960, 1962), and perhaps a similar explanation holds for them.

The  $T_{th}$  of calopterygids perched or in flight has not been measured, but in *Micrathyria atra*, a small tropical libellulid,  $T_{th}$  is sometimes 4-5°C above  $T_a$  when perched in the sun (MAY, 1985), while in the damselfly, *Argia difficilis*, there may be a temperature excess of 7°C (SHELLEY, 1982; MAY, 1991). It is likely that *C. haemorrhoidalis* experiences a similar  $T_{th}$  excess when perched in the sun at  $T_a$  30°C, which would mean that the  $T_{th}$  is close to the value at which spontaneous wingclaps appear. Thus short flights (<1 s) in which not much forward speed is attained may raise the temperature above the wingclap threshold. That wingclapping is not more pronounced after longer flights, particularly after escalated conflicts, may be because the higher flight speeds provide more cooling. In some butterflies  $T_{th}$  may be higher when perched than when in flight (HEINRICH, 1986).

WEIS-FOGH (1967) made measurements of the thoracic volume changes which accompany wing movements in aeshnid dragonflies. In this family the wingbeat cycle is accompanied both by relatively large lateral movements of the pleura which are connected together by elastic ligaments at the dorsal carina, and by dorso-ventral movements of the meso- and metanotum. These autoventilate the thorax during flight, providing up to 10  $\mu$ l air stroke-1 through open spiracles (MILLER, 1962). The volume of air displaced by wing movements in *C. splendens* is about 1.4  $\mu$ l stroke-1 with a wing amplitude of 135°, or 14  $\mu$ l air g-1 stroke-1, which compares favourably with the 12  $\mu$ l g-1 stroke-1 found in *Aeshna mixta* with a wingstroke amplitude of 80° (WEIS-FOGH, 1967). The slow wing depression and fast elevation which characterise wingclapping may allow inspired air to equilibrate with the internal temperature before being expired. If wingclapping cooled the thorax mainly by external air movement, as suggested by ERICKSON & REID (1990), a fast depression and elevation would be more effective.

The persistence of larval respiratory responses into the teneral adult stage is



of considerable ontogenetic interest. It may reflect the rapidity of metamorphosis of Odonata, in which certain larval central-nervous mechanisms, not required by the adult, initially persist but then become inactive after the first 24 h of adult life. Similarly I have observed teneral *Enallagma cyathigerum*, when suffering from oxygen shortage, to make lateral abdominal movements comparable to those of hypoxic larvae when they wag the abdomen. The linkage of shaking movements with wingclapping provides further evidence that wingclapping may serve a respiratory as well as a thermoregulatory function.

If thermoregulatory and respiratory functions are served by wingclapping, why does wingclapping not occur in other odonate families? The answer may be that there is a much more effective abdominal ventilating system in Anisoptera than in Zygoptera, which can ventilate the thorax adequately after flight (MILLER, 1962). In those Zygoptera which are smaller than calopterygids, by contrast, there is probably less heat accumulation or oxygen deficiency during flight because of the relatively large surface-area to volume ratio, which makes additional heat-dissipating or ventilating systems unnecessary.

In conclusion I propose that wingclapping in calopterygids, in addition to possible behavioural roles, serves both thermoregulatory and respiratory functions. Cooling may be achieved by moving air externally over the thorax, as suggested by ERICKSON & REID (1990), and in addition by circulating it within the pterothoracic tracheal system where it can also improve the oxygen supply. I suggest that such a system is found only in calopterygid species because of their relatively large size but inefficient abdominal pumping system.

#### REFERENCES

- BICK, G.H. & J.C. BICK, 1965. Demography and behavior of the damselfly *Argia apicalis* (Say) (Odonata: Coenagrionidae). *Ecology* 46: 461-472.
- BICK, C.H. & J.C. BICK, 1978. The significance of wingclapping in Zygoptera. *Odonatologica* 7: 5-9.
- CASEY, T.M., 1988. Thermoregulation and heat exchange. *Adv. Insect Physiol.* 20: 119-146.
- ERICKSON, C.J. & M.E. REID, 1990. Wingclapping behaviour in *Calopteryx maculata* (P. de Beauvois) (Zygoptera: Calopterygidae). *Odonatologica* 18: 379-383.
- HEINRICH, B., 1986. Comparative thermoregulation of four montane butterflies of different mass. *Physiol. Zool.* 59: 616-626.
- HEYMER, A., 1973. Beiträge zur Ethologie und Evolution der Calopterygidae. *J. comp. Ethol.* (Suppl.) 11: 1-94.
- MAY, M.L., 1985. Thermoregulation. In: G.A. Kerkut & L.I. Gilbert, [Eds], *Comprehensive insect physiology, biochemistry and pharmacology*, vol. 4, pp. 507-552, Pergamon Press, Oxford.
- MAY, M.L., 1991. Thermal adaptations in dragonflies, revisited. *Adv. Odonatol.* 5: 71-88.
- MILL, P.J., 1985. Structure and function of the respiratory system. In: G.A. Kerkut & L.I. Gilbert, [Eds], *Comprehensive insect physiology, biochemistry and pharmacology*, vol. 3, pp. 517-593, Pergamon Press, Oxford.
- MILLER, P.L., 1960. Respiration in the desert locust. III. Ventilation and the spiracles during flight. *J. exp. Biol.* 37: 264-278.

- MILLER, P.L., 1962. Spiracle control in adult dragonflies. *J. exp. Biol.* 39: 513-535.
- MILLER, P.L., 1992. The effect of oxygen lack on egg hatching in an Indian dragonfly, *Potamarcha congener*. *Physiol. Ent.* 17: 68-72.
- MILLER, P.L., 1993. Responses of rectal pumping to oxygen lack by larval *Calopteryx splendens* (Zygoptera: Odonata). *Physiol. Ent.* 18: 379-388.
- RUPPELL, G., 1985. Kinematic and behavioural aspects of flight of the male banded Agrion, *Calopteryx (Agrion) splendens* L. In: M. Gewecke & C. Wendler, [Eds], *Insect locomotion*, pp. 195-204. Parey, Berlin.
- RÜPPELL, G., 1989. Kinematic analysis of symmetrical flight manoeuvres in Odonata. *J. exp. Biol.* 144: 13-42.
- SHELLEY, T.E., 1982. Comparative foraging behaviour of light- versus shade-seeking adult damselflies in a lowland neotropical forest (Odonata: Zygoptera). *Physiol. Zool.* 55: 335-343.
- WAAGE, J.K., 1984. Female and male interactions during courtship in *Calopteryx maculata* and *C. dimidiata* (Odonata: Calopterygidae): influence of oviposition behaviour. *Anim. Behav.* 32: 400-404.
- WAAGE, J.K., 1988. Reproductive behaviour of the damselfly *Calopteryx dimidiata* Burmeister (Zygoptera: Calopterygidae). *Odonatologica* 17: 365-378.
- WEIS-FOGH, T., 1967. Respiration and tracheal ventilation in locusts and other flying insects. *J. exp. Biol.* 47: 561-587.
- ZAHNER, R., 1959. Ueber die Bindung der mitteleuropäischen *Calopteryx*-Arten (Odonata, Zygoptera) an den Lebensraum des strömenden Wassers. *Int. Revue ges. Hydrobiol.* 44: 51-130.