Adv. Odonatol. 6 : 79-88 April, 1994

SUBMERGED OVIPOSITION AND RESPONSES TO OXYGEN LACK IN *ENALLAGMA CYATHIGERUM* (CHARPENTIER) (ZYGOPTERA : COENAGRIONIDAE)

P. L. MILLER

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

Female *Enallagma cyathigerum* (Charpentier) regularly oviposit below the water surface on submerged vegetation. While submerged they probably depend for oxygen on an air layer trapped on the body surface which is thought to act as a physical gill. Although they may attempt to maximise egg-laying during each period of submergence because of high predation and low survival rates, oxygen shortage is one factor which may cause them to surface while still containing some ripe eggs. Females float up and most become trapped in the meniscus where they depend on males for their rescue. Rescuing males are rewarded if females contain some ripe eggs, accept copulation and return to oviposit soon afterwards. The examination of females caught at the surface immediately after oviposition showed that 51% contained at least 10 ripe eggs in the lateral oviducts and 28% contained > 50 eggs. Females may attempt to prolong their submergence by making body-rolling movements which enhance the oxygen supply through stirring the water in contact with their superficial air layer. These movements bear some resemblance to the shaking behaviour of hypoxic *Calopteryx* larvae, and the possibility of their common origin is discussed.

INTRODUCTION

Adult female damselflies in several genera including *Calopteryx*, *Erythromma*, *Enallagma* and *Lestes* spp. sometimes descend below the surface to oviposit on submerged plants, doing so for periods of up to an hour (CORBET, 1962 ; BICK & BICK, 1963 ; FINCKE, 1986 ; MILLER, 1990). They thus re-enter the larval habitat and may encounter conditions for which larval adaptations are appropriate. It is of some interest therefore to search for larval responses which are retained in adults, for example those which might be triggered by oxygen shortage. Some observations have therefore been made on the larvae and adults of *Calopteryx splendens* (Harris), *Enallagma cyathigerum* (Charpentier) and other species with this possibility in mind.

Larval Anisoptera respond to oxygen shortage by increasing the rate of rectal pumping and by locomotion towards the surface. Zygopteran larvae show a number of responses to hypoxia which include the lateral waving of the abdomen and lamellae, approaching the water surface, exposing the first pair of spiracles to the air (in last larval instars), spreading the wing cases and lamellae, and, in *Calopteryx* spp., shaking the body rapidly from side to side (*Rütteln*) (ZAHNER, 1959 ; CORBET, 1962 ; MILL, 1974, 1985) and increasing the rate of rectal pumping (MILLER, 1993).

During submerged oviposition female *E. cyathigerum* may experience oxygen shortage which causes them either to release their grip and float to the surface or to make body-rolling movements. The latter stir the surrounding water and may enhance gaseous exchange across the thin air layer which is trapped on some parts of their body surface and which communicates with the spiracles.

The rescue of female *E. cyathigerum* after oviposition, together with the potential rewards for the rescuing males, are first considered and the body-rolling response in hypoxic adults is then described. This response bears some resemblance to the shaking of hypoxic *Calopteryx* larvae, and the possibility of a common origin is discussed.

THE RESCUE OF FEMALES AFTER SUBMERGED OVIPOSITION

Observations have been carried out on *E. cyathigerum* at gravel pits near Oxford, U.K., since 1989. Under favourable conditions tandem pairs fly over the water surface up to 100 m from the margins of the habitat in regions where they can locate submerged vegetation (*Elodea, Potamogeton, Myriophylla* spp.), seeking stems which reach the surface on which females can climb down. Males normally release the tandem clasp before their thorax is submerged, but if strongly harassed by other males, they may not do so until they have been pulled 5-10 cm below the surface by the female. Other males, clinging to the female, may also occasionally become submerged in this way.

Females which had submerged only briefly or had been experimentally held under water for 10-20 s were found usually to be able to break through the meniscus and fly off immediately after surfacing. In contrast those which had been forcibly submerged for longer or had surfaced naturally after oviposition usually became trapped in the meniscus and were incapable of flight. Apparently the cuticle becomes gradually more wetted during a long dive, and the female may also be weakened by prolonged submergence.

On surfacing most females are able to open their forewings above the water and beat them. This is because they have been kept dry between the hind wings during a dive. Forewing beating can successfully propel a female along the surface for at least 20 m at up to 5 cm s-1, inspite of the considerable drag offered by the laterally spread hindwings and the body, and the female can steer towards the bank or emergent vegetation where she can climb out, the progress resembling that of a slow hovercraft. But although escape from the water surface is occasionally possible without male assistance, many females are caught by fish {mainly fingerling pike, *Esox lucius* (Linnaeus)} as they struggle there, or they are blown out by the wind and drown. Sometimes a rescuing male is unable to lift a female and she may then assist the male to tow her through the water by forewing beating (Fig. 1). Successful tows of up to 30 m have been witnessed.

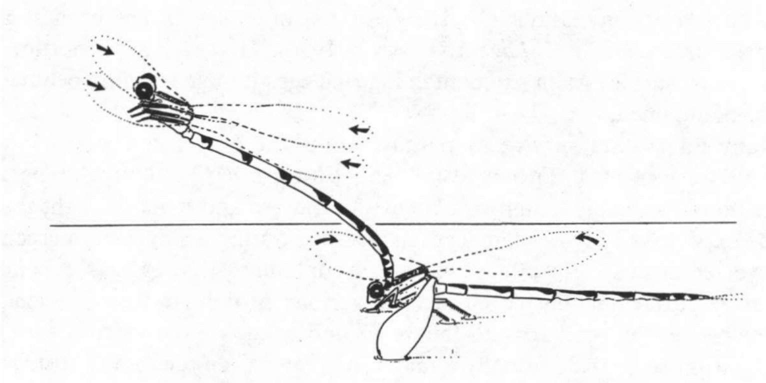


Fig. 1. A male is shown towing a female along the water surface, with the female assisting by beating her forewings while the hindwings are spread out in the water.

In 1989 less than 2% (2/126) of female *E. cyathigerum* which were observed to surface spontaneously after oviposition were capable of unassisted take-off and flight towards the bank, the remaining 98% being trapped in the meniscus. On July 5th, when there was a light offshore wind, of 77 rescue attempts which were observed, 33% failed, the females being eventually abandoned, 64% were successful with males flying to reeds with their captives, while a further 3% were also successful, the males towing their females along the water surface to the margin. On 6th July, when there was no offshore wind, of 49 observed rescue attempts, 18% were unsuccessful, and 71% were successful by flight and 10% by towing (MILLER, 1990).

FINCKE (1986) found that 81% of *E. hageni* could take off from the surface after oviposition without the assistance of males, though many might subsequently be caught by males in the air. The large difference in numbers (2%

and 81%) of *E. cyathigerum* and *E. hageni* capable of immediate take-off after oviposition is puzzling: it could be due to differences in the condition of the females, for example depending on their metabolic reserves or the wettability of surface waxes, or on the duration of the preceding submergence, the temperature, or even to differences in the surface tensions of the two habitats resulting from different amounts of organic decay.

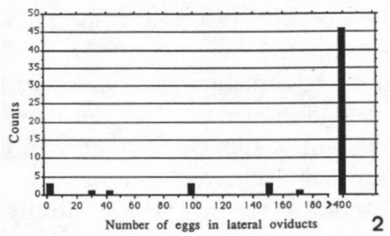
REWARDS FOR RESCUING MALES

Males are rewarded for rescuing females only if the females, 1) contain some ripe eggs, 2) accept copulation, and 3) oviposit shortly afterwards (FINCKE, 1986). Males cannot apparently determine a female's eggload and may thus rescue some females containing no mature egg. Such females are likely to refuse copulatory invitations (N. HUMPHREYS, unpubl.), as has been shown to be the case also in *E. hageni* (FINCKE, 1986). However a proportion of females must surface when still containing ripe eggs if male rescuing behaviour is to be maintained.

Many damselflies survive as mature adults for only a few days (PARR, 1973, 1976; BANKS & THOMPSON, 1985; FINCKE, 1982, 1988). Submerged oviposition is probably a high-risk behaviour pattern and females might therefore be expected to lay as many eggs as possible during a single submergence. However some females surface while still containing ripe eggs (see below). Premature surfacing may be caused by various proximate factors such as, 1) displacement by predators, 2) failure to find adequate oviposition sites, or 3) oxygen shortage. Additionally females may have been selected to distribute their eggs widely during the course of several dives.

The total clutch size in *E. hageni* is 400-500 eggs (FINCKE, 1982, 1986. Cf. THOMPSON, 1990; GRIBBIN & THOMPSON, 1990; CORDERO, 1991), and FINCKE found an average deposition rate of 13 eggs min-1. With an average dive duration of 18.4 mins., about half a clutch could be laid per dive. According to FINCKE females were more likely to accept copulations from rescuing males when they had been submerged for 10 min or less, whereas most refused them after submergence of 19.5 min or more, indicating that the number of unlaid eggs probably determined their receptivity.

Counts of the number of ripe eggs in the lateral oviducts of 58 female *E. cyathigerum* which had been caught in copulation before oviposition showed that 79% females contained full egg loads (400-500 eggs) (Fig. 2). However counts made on 130 females collected as they surfaced after oviposition showed that 45% surfaced with no egg in the lateral oviducts, while 51% contained 10 or more eggs and 28% contained >50 eggs (Fig. 3). In addition many females contained mature eggs in the terminal portions of the ovarioles. If these were soon to be released into the lateral oviducts, a male



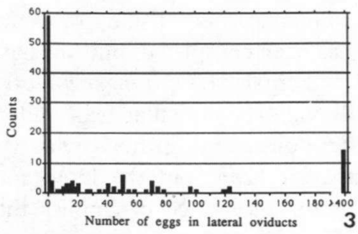


Fig. 2. Histogram of the counts of eggs in the lateral oviducts of 58 females caught in tandem or copulation near water before oviposition.
Fig. 3. Histogram of the number of eggs in the lateral oviducts of 130 females caught after they had spontaneously surfaced.

in tandem might gain more fertilisable eggs by delaying oviposition with a receptive female (Cowhig, unpubl.).

In conclusion more than a quarter of surfacing females contain > 50 ripe eggs in the lateral oviducts and many also had some ripe eggs in the ovarioles. If such females accepted copulations, their rescuers might be able to fertilise most of the eggs laid in the ensuing bout of oviposition. Attempts to measure acceptance rates among rescued females are now being made.

RESPONSES TO OXYGEN SHORTAGE

The responses of adult dragonflies to hypoxia include spiracular opening and hyperventilation, with various combinations of spiracles becoming coupled to the ventilatory movements according to the species (Miller, 1962), and with a threshold partly determined by the insect's water balance (Miller, 1964). In addition, body-rolling movements may be performed by submerged damselflies when they become hypoxic, as is described below.

In species which oviposit after submergence, females depend for their oxygen supply on an air layer trapped on some parts of the body and wing surfaces by surface waxes and bristles. It is presumed to act as a physical gill allowing oxygen to diffuse in from the water while nitrogen and carbon dioxide simultaneously diffuse outwards. The time for which a physical gill can function before it must be renewed at the surface is limited, depending on several factors including the size of the gill, the temperature, the depth of the dive, the oxygen tension in the surrounding water and the rate of oxygen consumption. In some water bugs (e.g. *Notonecta*) it can increase by up to eight times the oxygen available for respiration during a dive (Thorpe, 1950; Rahn & Paganelli 1968; Mill, 1974). Visits to the surface for more air may be excited by the activity of superficial mechanoreceptors which detect volume reductions of the physical gill, or by internal receptors responding to oxygen lack. Similar mechanisms may cause ovipositing damselflies to commence body-rolling movements or to seek the surface before oviposition has been completed, but this has not yet been explored.

Female *E. cyathigerum* do not appear to be structurally modified for diving. The cuticular wax and bristles which hold the air layer are not more developed than in non-diving species. Probably most spiracles can open into the air layer, but the large anterior thoracic pair (spiracles 1) is of major importance since it supplies the head and thoracic nervous system directly (MILLER, 1962). During submergence, abdominal expiration can sometimes be observed to pump air from the tracheal system into the superficial layer causing it to expand, while the ensuing inspiration sucks air in from the external layer. The wax layer and bristles are easily damaged and the air layer may in consequence be disrupted when a female descends below the surface after being handled, giving rise to rapid asphyxiation if she is unable to regain the surface.

Most observed oviposition by *E. cyathigerum* was into the green stems of *Elodea canadensis* and *Potamogeton pectinatus*, regions where high oxygen levels are to be expected resulting from photosynthesis. Some took place into the roots of willow trees (*Salix* spp.) or the stems and roots of *Sparganium erectum, Typha latifolia* and other plants — regions where oxygen may be less abundant. Even in well-oxygenated water a physical gill has a limited life and a submerged insect must soon return to the surface to renew it. A female *E. cyathigerum*, experiencing hypoxia, may either release her hold and float to the surface or attempt to prolong a dive by ventilating the physical gill. Body-rolling movements which appear under such circumstances are brought about by flexion and extension of the legs at the coxal joints on alternate sides while the tarsi maintain their grip (Fig. 4). These cause the body to rotate about its long axis through up to 20° in each direction while at the same time the abdomen makes lateral yawing movements.

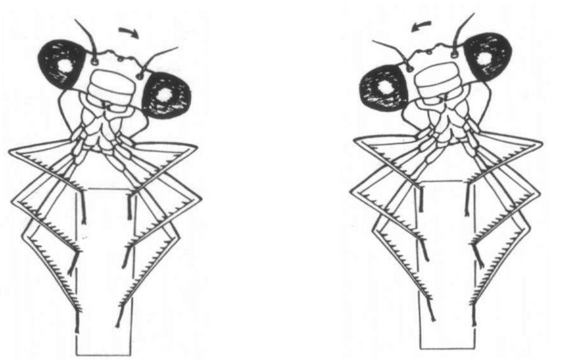


Fig. 4. Successive positions of a zygopteran adult in which body-rolling has been excited by hyoxic conditions.

Rolling movements can be made to occur by holding damselflies under water or by perfusing them out of water with hypoxic gases. When damselflies were trapped under a grid in previously boiled water, rolling movements started in some individuals in < 4 min. If the tarsi lost their grip, abrupt and synchronised coxal flexions on one side could be seen to be accompanied by extensions on the other, and they were sometimes of greatest amplitude and occurred slightly earlier in the second pair of legs than in the remainder. Alternatively weak activity might be confined to the second legs alone, suggesting a leading role for the mesothoracic ganglion in organising the activity.

When perfused with 2% oxygen in nitrogen out of water, some damselflies showed continual body-rolling activity for up to an hour at frequencies of 96-120 min-1, while in others it was intermittent or might not appear atall. It was sometimes accompanied by abdominal probing movements resembling oviposition which alternated with short bouts of walking. Rolling and walking are clearly incompatible activities.

Body-rolling has occasionally been observed in nature in female *E. cyathigerum* ovipositing in shallow water. It has not been seen to occur out of water except under experimental conditions. It cannot be elicited in all individuals tested, and is more readily evoked in females than males (Tab. I). It occasionally occurs in some other species, including *Ischnura elegans* whose oviposition normally takes place at the surface.

Table I

Numbers of damselflies showing body-rolling response in 2% oxygen

	Females	%	Males	%
Enallagma cyathigerum	20/42	48	3/40	8
Erythromma najas	9/24	38	3/25	12
Ischnura elegans	4/12	33	1/25	4

DISCUSSION

Submerged oviposition probably evolved as a means of exploiting additional oviposition sites in underwater plants. In *E. cyathigerum* a male guards the point of descent of his female only briefly and other males commonly hover close to a submerging female. Submerged oviposition not only prevents harassment by other males but it also liberates males from the long periods of contact guarding during oviposition, which are features of many other coenagrionids. Males thus gain from submerged oviposition since they are immediately available for further mating opportunities while their previous female is not accessible to rival males.

Females leave the surface and reach their oviposition sites by rapidly scurrying down plant stems and leaves, thereby escaping from male harassment. They are unable to swim and when they release their underwater grip they quickly float to the surface as a result of their relatively voluminous tracheal system and the adhering air layer. As they ascend they make characteristic abdominal bending movements which enhance their visibility and attract rescuing males. Females struggling at the surface excite rescue by males, whereas motionless individuals are at most inspected only briefly. Many drowned females, still afloat, could be found near actively used oviposition sites.

Rescuing behaviour is advantageous to males because some rescued females contain eggs, accept copulations and are prepared to oviposit again soon afterwards. Females which refuse copulation and are eventually released may sometimes make initial abdominal bending movements in response to copulatory invitations, as though in a marginally receptive state. These may serve to prolong the period of tandem when perhaps the female can assess the male, and during which her cuticle can dry out thoroughly.

High predation pressure and the uncertainty of rescue would be expected to have selected females to maximise egg-laying during each submergence. However females may sometimes surface before completing the laying of a clutch for several reasons one of which is oxygen shortage. As a result males can benefit from the further availability of receptive females. Momentary exposure at the surface would be adequate to renew a female's air layer and oviposition could be immediately resumed without the need for rescue if plants were available to crawl down on. However at many of the study sites, although submerged plants were abundant only occasional stems reached the surface and females commonly appeared far from surface vegetation. The large number of males which continually flew over such sites suggested that males derive considerable benefit from rescuing females.

The body-rolling response of adult damselflies bears some resemblance to the shaking movements of hypoxic *Calopteryx* spp. larvae. (ZAHNER, 1959). In both there are similar leg movements while the tarsi maintain their grip and both are thought to serve respiratory needs. However shaking is always performed rapidly in short bursts and it moves the body from side to side while body-rolling is slower, may occur continually and consists mainly of longitudinal rotating movements. Teneral adult *C. splendens* may be excited by hypoxia to perform shaking movements very similar to those of the larvae, but such movements have not been seen in mature individuals. It would not be suprising if some larval responses persisted into the adult stage since the transition from an aquatic to a terrestrial environment is abrupt while time is needed to readjust the underlying neural circuitry. However if body-rolling in adult *E. cyathigerum* has developed from a larval response resembling the shaking of *Calopteryx* spp., we must assume that it has been altered to meet adult needs and perhaps suppressed in coenagrionid larvae in favour of alternative methods of irrigating the gills.

ACKNOWLEDGEMENTS

I am most grateful to Margot Cowhig and Natalie Humphreys for their diligent assistance in the field and laboratory.

REFERENCES

Banks, M. J. & D. J. Thompson, 1985. Emergence, longevity and breeding area fidelity in Coenagrion puella (Zygoptera : Coenagrionidae). *Odonatologica* 14 : 279-286.

Bick, G. H. & J. C. Bick, 1983. Behaviour and population structure of the damselfly Enallagma civile (Hagen) (Odonata : Coenagriidae). *Southwest Nat.* 8 : 57-84.

Corbet, P. S., 1962. *A biology of dragonflies.* Witherby, London.

Cordero, A., 1991. Fecundity of Ischnura graellsii (Rambur) in the laboratory (Zygoptera : Coenagrionidae). *Odonatologica* 20 : 37-44.

Fincke, O. M., 1982. Lifetime mating success in a marginal population of the damselfly Enallagma hageni (Walsh) (Odonata : Coenagrionidae). *Behav. Ecol. Sociobiol.* 10 : 283-302.

Fincke, O. M., 1986. Underwater oviposition, male vigilance, and female multiple mating in a damselfly (Odonata : Coenagrionidae). *Behav. Ecol. Sociobiol.* 18 : 405-412.

Fincke, O. M., 1988. Sources of variation in lifetime reproductive success in a nonterritorial damselfly (Odonata : Coenagrionidae), p. 24-43. *In* : T. H. Clutton-Brock [Ed.], *Reproductive succes.* Univ. Chicago Press, Chicago & London.

Gribbin, S. D. & D. J. Thompson, 1990. Egg size and clutch size in Pyrrhosoma nymphula (Sulzer) (Zygoptera : Coenagrionidae). *Odonatologica* 19 : 347-357.

Mill, P. J., 1974. Respiration : aquatic insects, p. 403-467. *In* : M. Rockstein [Ed.], *Physiology of Insecta*, 2nd Ed., Vol. 6 ; Academic Press, New York & London.

Mill, P. J., 1985. Structure and function of the respiratory system, p. 517-593. *In* : G. A. Kerkut & L. I. Gilbert [Eds.], *Comparative insect physiology, biochemistry and pharmacology*, Vol. 3, p. 517-593. Pergamon Press, Oxford & New York.

Miller, P. L., 1962. Spiracle control in adult dragonflies (Odonata). *J. exp. Biol.* 39 : 513-535.

Miller, P. L., 1964. Factors altering spiracle control in adult dragonflies : water balance. *J. exp. Biol.* 41 : 331-343.

Miller, P. L., 1990. The rescue service provided by male Enallagma cyathigerum (Charpentier) for females after oviposition. *J. Br. Dragonfly Soc.* 6 : 8-14.

Miller, P. L., 1993. Responses of rectal pumping to oxygen lack in Calopteryx splendens (Zygoptera : Odonata). *Physiol. Ent.* (in press).

Parr, M. J., 1973. Ecological studies of Ischnura elegans (vander Linden) (Zygoptera : Coenagrionidae). II. Survivorship, local movement and dispersal. *Odonatologica* 2 : 159-174.

RAHN, H. & C. V. PAGANELLI, 1968. Gas exchange in gas gills of diving insects. *Resp. Physiol.* 5 : 145-164.
THOMPSON, D. J., 1990. The effects of survival and weather on lifetime egg production in a model damselfly. *Ecol. Ent.* 15 : 455-462.
THORPE, W. H., 1950. Plastron respiration in aquatic insects. *Biol. Rev.* 25 : 344-390.
ZAHNER, R. 1959. Uber die Bindung der mitteleuropaischen Calopteryx-Arten (Odonata, Zygoptera) an den Lebensraum des stromenden Wassers. *Internat. Rev. Ges. Hydrobiol.* 44 : 51-130.