Odonatologica 32(1): 29-37 *February 24, 2003*

REPRODUCTIVE MORPHOLOGY AND BEHAVIOUR IN *COENAGRION MERCURIALE* (CHARPENTIER) (ZYGOPTERA: COENAGRIONIDAE)

B.V. PURSE* and D.J. THOMPSON
Population and Evolutionary Biology Research Group, Nicholson Building, School of Biological Sciences, University of Liverpool, Liverpool, L69 3GS, United Kingdom
e-mail: beth.purse@bbsrc.ac.uk

Received April 30, 2002 / Revised and Accepted July 1, 2002

The reproductive morphology and behaviour of ♂ and ♀ at the northern edge of the sp. range, in Britain are described. Copula duration was relatively short and occurred in tussocks around the stream and oviposition usually occurred in tandem. Although there was no significant relationship between body size and clutch size, large clutches were only found in the largest individuals and larger ♀♀ produced larger, and thus better-provisioned eggs. Examination of the dimensions of the aedeagus and the ♀ sperm storage organs revealed that ♂♂ could remove rival sperm from both the bursa copulatrix and spermatheca consistent with the presence of extensive proximally oriented microspination with sperm masses on the surface of the aedeagi. ♀♀ store sperm from previous matings and a few partition clutches between consecutive oviposition episodes. The fulfilment of these ecological and behavioural pre-requisites and the predominance of mate guarding during oviposition suggests that sperm competition by sperm displacement is prevalent in this sp. and constitutes a substantial selection pressure.

INTRODUCTION

Coenagrion mercuriale (Charpentier) has a south-western distribution in Europe (37-49°N, 3-12°E) (ASKEW, 1988) and reaches its northern range margin in Britain (51°N, 1°W) (PURSE, 2001) where it is listed as rare (category 3 in the Red Data Book). It is listed in Appendix II of the Berne Convention on the Conservation of European Wildlife and Natural Habitats (1979), which outlaws the collection or possession of listed species. It is also listed in Annex II of the European Habitat and Species Directive (1992) that requires that special areas of conservation should be

---

* **Present address:** Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Surrey, GU24 0NF, United Kingdom

designated. In Britain, *C. mercuriale* occurs in small populations and its breeding habitat is very patchily distributed (PURSE, 2001).

In a recent study of lifetime mating success in a marginal population of *C. mercuriale* (B. V. Purse, unpublished), activity of both males and females was strongly influenced by variation in weather conditions, limiting the opportunity for sexual selection. In this paper, additional aspects of the reproductive behaviour of *C. mercuriale* are examined, including, clutch size and partitioning, and aedeagus morphology, to infer whether variation in fecundity or fertilisations may be important components of reproductive success.

SPECIES AND STUDY SITES

Coenagrion mercuriale breeds in two rare habitat types in Britain – small spring fed streams on lowland heathland and old water meadow ditch systems on chalk rivers (BROOKS & LEWINGTON, 1997). It is a non-territorial, scrambling damselfly. Suitable oviposition habitat, consisting of mats of soft-stemmed herbs, e.g. *Hypericum elodes* and *Potamogeton polygonifolius*, is usually sparsely distributed along streams or ditches. Tussocks of vegetation of medium height (0.2-0.6 m) surrounding the stream are used during the maturation period and for roosting and feeding of mature adults. In this study, behaviour of sexually mature adults was examined in populations on two heathland sites. Aylesbeare Common, East Devon (SY 055907) contains a small, isolated population (< 230 individuals in 1998) on a basic flush system on a south-facing slope (KERRY, 1994). Crockford, Beaulieu Heath, Hampshire (SU 350990) contains a large population (>2000 individuals in 1998) on a stream running through valley mire and wet heath.

METHODS

CLUTCH SIZE, EGG SIZE AND OVIPOSITION BEHAVIOUR. – Females were collected from Upper Crockford in 1999 - (1) before copulation in the morning, (2) after copulation and (3) after oviposition - and retained in 70% ethanol. In the laboratory, head width (x 20 magnification) and the length of a portion of the left forewing between the pterostigma and the point of attachment with the body were measured under a binocular dissecting microscope (x 10) calibrated using a slide micrometer and eyepiece graticule. The spermatheca and bursa were removed from each female and observed under the microscope to score sperm presence, visible as a grey mass, inside them. The total number of mature eggs inside the ovaries was counted. These could be distinguished from immature eggs by their larger size, lighter colouration and position. The latter are concentrated towards the anterior of the ovarioles. Ten mature eggs, selected at random from each female, were measured under a dissecting microscope as above (x 30). With regards to oviposition behaviour, females in tandem generally settled on the substrate while males either hovered upright on the prothorax of the female or perched on vertical structures above the female. Upon landing the female would curve her abdomen to bring her ovipositor in contact with the plant substrate. Often this contact lasted less than five seconds before the abdomen was held out straight again or rapid probing movements were performed with the ovipositor in a variety of positions until a 'suitable' position was found. Thus an oviposition bout was defined as a period in excess of five seconds when the female's ovipositor was in contact with the plant stem. At Aylesbeare, observations were made on the number of times females oviposited entirely alone or instances where contact-guarding by males did not continue throughout oviposition. Any effect of predation or weather conditions on oviposition behaviour was noted. These are compared to similar observations made at Upper Crockford in 1999. Observations of the numbers of eggs laid by females during all bouts in an episode were made by carefully collecting all plant material into which they had oviposited. This plant material was put into tap water for 2-6 days. It was then transferred to a mixture of concentrated acetic acid and 96% ethanol in a 2:1 ratio for 24 hours to remove chlorophyll (Andreas Martens, pers. comm.). It was then cleaned in 96% ethanol before being preserved until dissection in 70% ethanol. Stems were dissected to remove the eggs (using micropyles protruding from the leaf surface as a guide to their location).

AEDEAGUS MORPHOLOGY. – Aedeagi were removed from several males of *C. mercuriale* using fine forceps, and freeze-dehydrated, coated with gold palladium and mounted according to the method of VELTCAMP et al. (1994). They were then examined under a scanning electron microscope.

RESULTS

In both Aylesbeare (n = 15) and Crockford (n = 22), copulations began and occurred in tussocks of vegetation near the stream rather than in areas of open water. Copula duration was

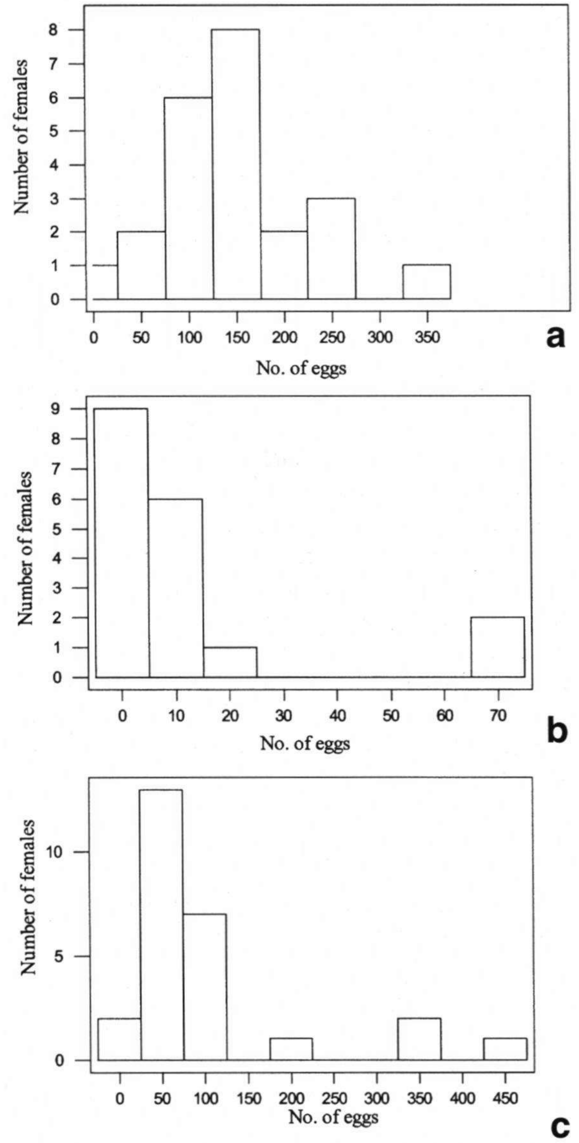

Fig. 1. Frequency distribution of number of eggs in *Coenagrion mercuriale*: (a) found in females before oviposition (n = 23); – (b) found in females after oviposition (n = 18); – (c) laid by females during oviposition focals (n = 27, all focals ended with a natural pair split) derived from plant stem dissections.

relatively short (Aylesbeare; $\mu \pm$s.e. = 19.5±0.6 min, n = 2: Crockford: $\mu \pm$s.e. = 23.1±1 min, range 14.2-45.0, n = 22). There was no effect of temperature on copula duration controlling for time of day (partial correlation coefficient r_p = -0.104, p = 0.662, n = 18). There was no effect of time of day on copula duration controlling for temperature (r_p = -0.273, p = 0.244, n = 18).

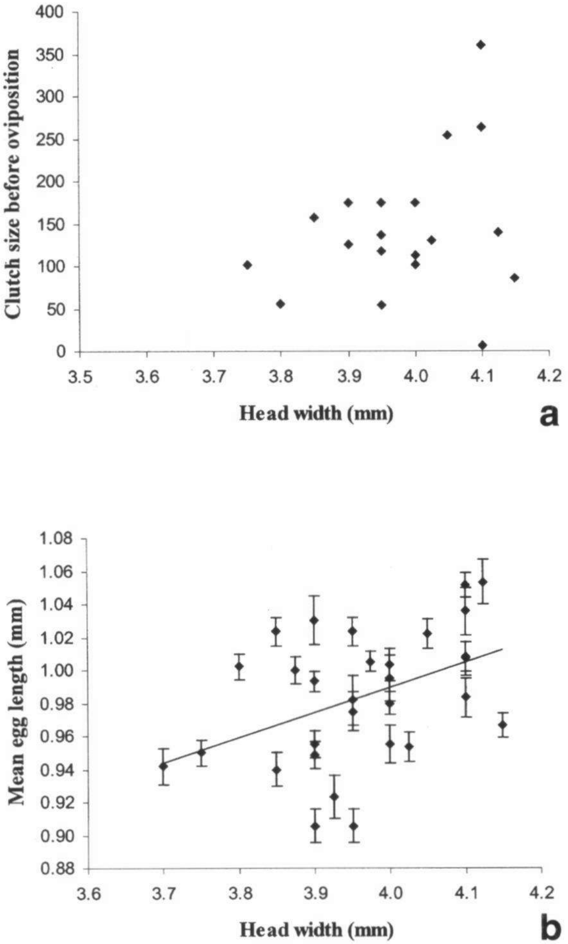

Fig. 2. Relationship between head width and (a) clutch size before oviposition (n = 20) and (b) mean egg length (± s.e.) (n = 32) in *Coenagrion mercuriale.*

For five pairs at Crockford, observed from the point at which they formed a tandem until the point at which they split after oviposition, the mean duration of the tandem was 69.9±16.0 minutes (range: 43-130.4). For nine pairs at Crockford, the time, between the beginning of copulation and the end of oviposition, spent in (1) copulation (2) tandem between copulation and oviposition and (3) ovipositing/searching for oviposition sites was scored and the mean and range of the duration of each in minutes was as follows: μ_1 = 24.1±2.4, range = 15.0-40.0; μ_2 = 13.3±3.5, range = 3.0-39.0; μ_3 = 30.5±5.5, range = 8.3-58.0. Copulation and oviposition, or searching for oviposition sites, each made up around 40% of a pair's time whilst 20% of it was spent in tandem in between copulation and oviposition. Of the time spent in behaviour (3), the mean percentage time spent actually ovipositing rather than searching for sites was 50.1±8. 5% (ranging from 23.5 to 100%).

In Aylesbeare, in 11 of the 15 oviposition episodes observed oviposition behaviour occurred in tandem, in two (13%) females oviposited entirely alone, and had not mated previously on the same day, and in two (13%) oviposition began in tandem but ended with the female ovipositing alone. Two ovipositions in Aylesbeare were prevented, having begun, by wind conditions whilst three were terminated in Upper Crockford when one member of a pair became stuck in sundew, *Drosera* sp.

The total number of eggs contained by females collected in the morning before oviposition at Upper Crockford ranged from 7 to 360 (Fig. 1a), with a mean of 146.1 ± 16.0. The frequency distribution of number of eggs found inside females after oviposition is shown in Figure 1b. The mean number of eggs left after oviposition was 12.7±4.9 but two individuals had more than 70 eggs remaining. This indicates that a small proportion of females may not lay all their eggs during one oviposition episode but may divide them between two episodes. This is supported by the fact that a substantial portion of females observed in pair focals laid 50 eggs or less in an oviposition episode (Fig. 1c).

Whilst there is no positive linear relationship between clutch size before oviposition and head width ($F = 1.80, p = 0.197, R^2 = 0.091$, d.f. = 19), maximum clutch size may be limited by body size i.e. large clutch sizes (>200 eggs) only occurred in individuals with large head widths (>4mm). Mean egg length was 0.981±0.003mm (ranging from 0.833-1.133, $n = 325$). There was a positive relationship between mean egg length and head width (Fig. 6.14, $F = 7.82, p = 0.009, R^2 = 0.207$, d.f. = 31).

All four females collected immediately after copulation had sperm in both storage organs. All but one of the fifteen females collected after ovipositon still had sperm in both their spermatheca and bursa indicating that the sperm store is not substantially depleted during one oviposition episode. Consistent with this, 8 out of 12 females collected before copulation contained sperm in one or both of the storage organs and thus sperm is stored between matings in *C. mercuriale*. The four females containing no sperm before copulation are assumed to have been virgin females.

Figure 3 shows the female reproductive tract of *C. mercuriale*. As in other odonates, two flat sclerotized plates bound the anterior region of the vagina dorsoventrally. In *C. mercuriale*, the outer surface of each plate has a collection of approximately 40 black circular structures, campaniform sensillae that are related to the coordination of sperm ejection during egg-laying (MILLER, 1987). The mean width of this region of the vagina was 393±7 μm (ranging from

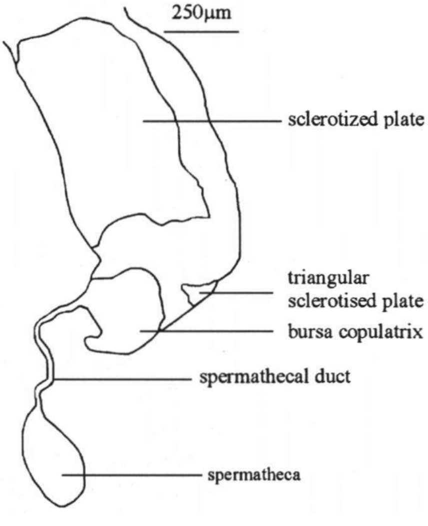

Fig. 3. The female reproductive system in *Coenagrion mercuriale*; scale bar represents 250 μm.

374 to 426, $n = 8$) whilst the mean length was 632±3 µm (ranging from 542 to 779). The spermatheca is well defined and joined to the bursa copulatrix via a narrow duct. This spermathecal duct had a mean length of 399±9 µm (ranging from 368 to 447, $n = 10$) and was 21±1 µm wide on average (range 16-24). The spermatheca was ovoid in shape with a mean width of 151±6 µm (ranging from 113 to 208, $n = 17$) and a mean length of 253±7 µm (ranging from 211 to 303). The bursa copulatrix had a mean width of 242±8 µm (ranging from 203 to

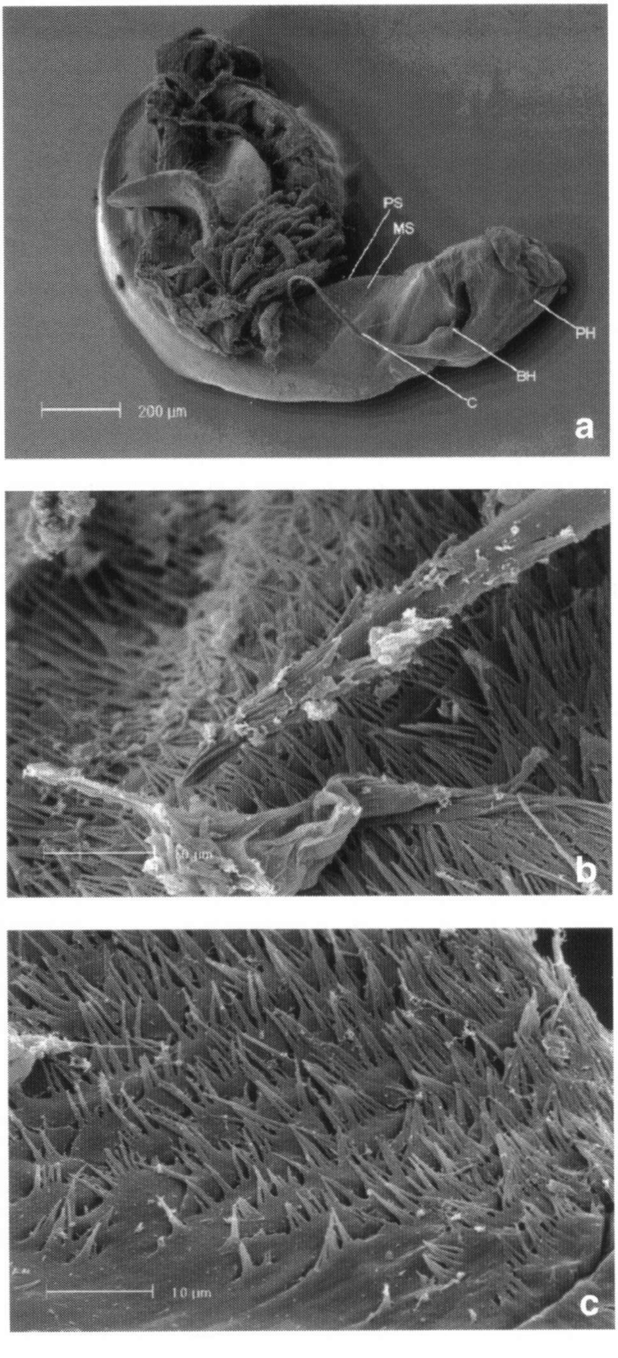

Fig. 4. *Coenagrion mercuriale*: (a) lateral view of the entire penis showing the penis shaft (PS) with the region of microspination (MS) and the extensible penis head (PH) with stout basal hooks (BH) and long, recurved cornua (C); – (b) tip of cornua with proximally-oriented spines, under which sperm masses are trapped. Background shows comb-like spines on dorsal surface of the penis shaft; – (c) dense overlapping layers of comb-like microspination on the dorsal surface of the penis shaft.

284, $n = 11$) and a mean length of 263±12 µm (ranging from 179-311). Thus the volume of the spermatheca is approximately half that of the bursa copulatrix and thus it probably stores up to a third of the total sperm load. In addition, near the proximal end of the bursa at the side of the vagina, there is a triangular sclerotized plate of unknown function.

Within the Zygoptera four types of penes have been recognised (WAAGE, 1986). Since the penis of *C. mercuriale* cannot be easily classified into one of these types, a detailed description of its form in relation to possible sperm removal function is necessary (see also KUHN, 1989). The distal segment of the aedeagus has an extensible head consisting of a flap of cuticle which folds back along the ventral surface of the penis. This flap (Fig. 4a) has two long, recurved horns or cornua, ranging from 478.0 to 561.5 µm in length, and a pair of stout hooks at its base (50 µm long). In addition, there is a membranous groove for sperm transfer on the dorsum of the penis head. Proximally--oriented micro-spination was evident on several areas of the penis. Firstly, long spines were found on the distal ends of the cornua that were approximately 4-5 µm (Fig. 4b). Shorter spines, around 2-3 µm in length, occur along the length of each flagellum and extensively over the ventro-lateral surface of the penis head at the base. Below the head, on the dorsal surface of the penis shaft, there are dense, overlapping layers of comb-like spines approximately 4 µm long (Fig. 4c), in the region marked as MS on Figure 4a. Sperm masses were found attached to all these sets of spines in male *C. mercuriale* clearly indicating their ability to remove sperm form the female sperm storage organs.

The width of the penis head where it attached to the penis shaft was 225 µm. It was 100-150 µm ($n = 2$) across between the tips of the basal hooks and 100-130 µm at the widest distal point before the head bifurcated to form the two cornua. The head was 353 µm long from its point of attachment to the penis shaft to the point of bifurcation. The cornua varied in width along their length ($n = 3$), ranging from 36-58 µm immediately after bifurcation at the head, from 11-15 µm along most of the length of the flagellum, tapering to 3-6 µm at the tip.

With regards to which portions of the male's penis have access to the female sperm storage organs during copulation, several inferences can be made. The bursa would accommodate the width of only the head and cornua the shaft being too wide. However the head is longer than the bursa (353 and 263 respectively) and so only a portion of the penis head could enter the bursa. The cornua are narrower over almost their entire length (11-15 µm after bifurcation) than the spermathecal duct (15-44 µm) and they are somewhat longer than this duct (478-561 µm versus 368-447 µm) such that they could enter the spermatheca of the female.

DISCUSSION

In another scrambling damselfly, FINCKE (1988) found that variation in fertilisations per mating was numerically much less important in determining lifetime reproductive success than mating components such as mating efficiency, oviposition efficiency and lifespan. Thus lifetime mating success constituted a good approximation to lifetime reproductive success. Although *C. mercuriale* is also a scrambling species, this study provides evidence that variation in fecundity and fertilisations may constitute important fitness components in this species.

Firstly, although there was no significant relationship between body size and clutch size for females, large clutch sizes were only found in the largest individuals indicating that maximum clutch size may be limited by space available in the abdomen (cf. BANKS & THOMPSON, 1987; GRIBBIN & THOMPSON, 1990). In addition, larger females produced larger eggs that may be better provisioned for embryonic and larval development, increasing offspring survivorship. This relationship arose in another coenagrionid, because both body size and egg length decreased over the season (GRIBBIN & THOMPSON, 1990). The range of egg length (from 0.833-1.133 μm) is similar to that of *Pyrrhosoma nymphula* (0.96-1.14 μm, GRIBBIN & THOMPSON, 1990).

Secondly, an examination of the aedeagus morphology indicated that males have access to almost the female's entire sperm store. The cornua of the penis was narrower and longer than the spermathecal duct which, in other odonates (A. Córdoba-Aguilar, unpublished, cited in MILLER, 1982; ROBINSON & NOVAK, 1997; WAAGE, 1979), is indicative of a male's ability to remove rivals' sperm from both the bursa and the spermatheca of the female (see also KUHN, 1989).

Extensive areas of proximally oriented microspination were also found on both the extensible head and the sheath of the aedeagus that contained masses of sperm. Females were found to store sperm from previous matings and did not show substantial sperm depletion after an oviposition episode. Some females did not lay their entire mature egg clutch in one oviposition episode and thus partitioned it between episodes and between males producing a substantial delay between insemination and fertilisation of eggs. This partitioning of a clutch between episodes may also occur in other coenagrionids where a low minimum clutch size is found in females collected before copulation e.g. *Pyrrhosoma nymphula* (68-754 eggs, GRIBBIN & THOMPSON, 1990). Thus all the ecological and behavioural pre-requisites necessary for sperm competiton by sperm displacement listed by WAAGE (1986) are satisfied in *C. mercuriale*, and the predominance of mate guarding throughout oviposition seen in this study suggests it constitutes a substantial selection pressure.

The lifetime mating success study on this species indicated that mating success was related positively to the time present at the breeding site and negatively to the proportion of the lifespan made up of bad weather (B.V. Purse, unpublished). Thus mating efficiency was assumed to be less important in marginal populations of scrambling species when activity is constrained by climatic variables. However, the sperm displacement ability and variation in egg size, clutch size and partitioning demonstrated here indicate that lifetime reproductive success may not be approximated by lifetime mating success in this species. Lifetime reproductive success studies that include variation in fecundity, fertilisation and offspring survivorship, as well as matings obtained, are required to confirm the relative importance of these components in core versus marginal populations.

ACKNOWLEDGEMENTS

BVP was supported by a Research Studentship provided by the Environment Agency, English Nature and the Countryside Council for Wales. Collection and dissection of *Coenagrion mercuriale* were carried out under licence from English Nature. BVP would like to thank TED MORROW for instruction in aspects of microdissection. We thank ANDREAS MARTENS for bringing to our attention the paper by J. Kuhn.

This paper is dedicated to Norman Moore; we know from his time in Dorset that *Coenagrion mercuriale* is one of his odonates!

REFERENCES

ASKEW, R.R., 1988. *The dragonflies of Europe*. Harley Books, Colchester.

BANKS, M.J. & D.J. THOMPSON, 1987. Lifetime reproductive success in females of the damselfly Coenagrion puella. *J. Anim. Ecol.* 56: 815-832.

BROOKS, S. & R. LEWINGTON, 1997. *Field guide to the dragonflies and damselflies of Britain and Ireland*. British Wildlife Publishing, Hampshire.

FINCKE, O.M., 1988. Sources of variation in lifetime reproductive success in a nonterritorial damselfly (Odonata: Coenagrionidae). *In*: T.H. Clutton Brock, [Ed.], *Reproductive success: studies of individual variation in contrasting breeding systems*, pp. 24-43, Univ. Chicago Press, Chicago.

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

KERRY, L., 1994. *Coenagrion mercuriale recovery programme in Devon, 1994*. English Nature, Peterborough.

KUHN, J., 1989. Spermienkonkurrenz bei mitteleuropäischen Zygoptera: eine Übersicht. *Verh. westdt. Entomol. Tag* 1988: 174-201.

MILLER, P.L., 1982. The occurrence and activity of sperm in mature female Enallagma cyathigerum (Charpentier) (Zygoptera: Coenagrionidae). *Odonatologica* 11: 159-161.

MILLER, P.L., 1987. An examination of the prolonged copulations of Ischnura elegans (Vander Linden) (Zygoptera: Coenagrionidae). *Odonatologica* 16: 37-56.

MILLER, P.L. & C.A. MILLER, 1981. Field observations on copulatory behaviour in Zygoptera, with an examination of the structure and activity of the male genitalia. *Odonatologica* 10: 201-218.

PURSE, B.V., 2001. *The ecology and conservation of the Southern Damselfly (Coenagrion mercuriale)*. PhD Thesis, Univ. Liverpool.

ROBINSON, J.V. & K.L. NOVAK, 1997. The relationship between mating system and penis morphology in ischnuran damselflies (Odonata: Coenagrionidae). *Biol. J. Linn. Soc.* 60: 187-200.

VELTCAMP, C.J., J.C. CHUBB, S.P. BIRCH, & J.W. EATON, 1994. A simple freeze dehydration method for studying epiphytic and epizoic communities using the scanning electron microscope. *Hydrobiologia* 288: 33-38.

WAAGE, J.K., 1979. Dual function of the damselfly penis: sperm removal and transfer. *Science* 203: 916-918.

WAAGE, J.K., 1986. Evidence for widespread sperm displacement ability among Zygoptera (Odonata) and the means for predicting its presence. *Biol. J. Linn. Soc.* 28: 285-300.