

**FIELD EXPERIMENTS ON
AGGREGATION BEHAVIOUR AND OVIPOSITION
IN *COENAGRION PUELLA* (L.)
(ZYGOPTERA : COENAGRIONIDAE)**

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Tandems of *C. puella* accumulate during oviposition. The presence of an ovipositing tandem attracts other pairs to the same site. Discrimination experiments show that a single motionless male in the typical vertical position of a tandem male stimulates aggregation and oviposition behaviour of other pairs. — In *Potamogeton natans* eggs are nearly always inserted into the basis of the leaf and the petiole, with a mean egg deposition rate of 7.07 eggs/min. During short stays no eggs are laid.

INTRODUCTION

In Zygoptera oviposition in tandem is common (WAAGE, 1984). The male guards the female while grasping her prothorax. In many species the male takes up a vertical motionless position on the female while she is sitting on the floating oviposition site. During windy weather he beats his wings to keep his balance, very rarely does he settle horizontally on the substrate. Tandems of these species are very unevenly dispersed at the water. Often conspicuous groups of egg laying pairs can be found, e.g. in *Aciagrion africanum* (LEMPERT, 1988), *Argia apicalis* (BICK & BICK, 1965), *A. moesta* (RÜPPEL *et al.*, 1987), *A. tibialis* (DUNKLE, 1990), *Cercion lindenii* (HEYMER, 1975), *Ceriagrion melanurum* and *C. nipponicum* (MIZUTA, 1988), *Platycnemis acutipennis* (HEYMER, 1974) and *Pyrrhosoma nymphula* (REHFELDT, 1990).

In *Coenagrion pulchellum* and *Platycnemis pennipes* groups develop because tandems land directly even at occupied oviposition sites (MARTENS,

1989, 1992b). The formation and causes of aggregation during oviposition are investigated in detail in *P. pennipes* (MARTENS, 1992b).

The aim of the following experiments is to demonstrate the considerable influence of conspecifics on the choice of oviposition sites in *Coenagrion puella*. Of particular interest is the possible relationship between the conspicuous mate-guarding position of the male and the formation of aggregations.

MATERIAL AND METHODS

Coenagrion puella is a medium-sized, sexually dimorphic damselfly. The body of the male is azure blue with black markings. The species is widely distributed throughout Europe and inhabits a wide range of waters, particularly small ponds (ASKEW, 1988).

The reproductive behaviour of *C. puella* has been described in detail by ROBERT (1958) and BANKS & THOMPSON (1985). Eggs are deposited into floating parts of various water plants, often in *Potamogeton* species (e.g. BANKS & THOMPSON, 1985; KLEIN, 1933; ROBERT, 1958; SCHIEMENZ, 1953; STARK, 1977; WESENBERG-LUND, 1913).

The study was carried out from 27 May to 15 June, 1989 and from 29 May to 1 June, 1990 at a ditch surrounding a man-made pond 15 km east of Braunschweig (Lower Saxony, Germany; 52°18'20"N, 10°46'20"E). At its southern part between two areas covered with *Potamogeton natans* discrimination experiments were carried out on a 10 m long section without water plants. This stretch had a width of 1.8–2.0 m and a maximal depth of 0.75 m. The steep banks were densely covered with *Juncus effusus* and *Scirpus sylvatica*. Other plants in and around the ditch were *Glyceria fluitans*, *G. maxima*, *Lemna trisulca*, *Carex* sp. and *Urtica dioica*. The whole water system, including the ditch, was constructed in 1980, and became rich in dragonflies and vegetation within a short space of time (MARTENS, 1983).

The experimental setup consisted of a pair of alternative substrates which was placed in the water. Two fresh floating leaves of *Potamogeton natans* were chosen to be as similar as possible and attached with metal clips to a submerged frame. The leaves were cut off with their 7 to 10 cm long petioles and fixed parallel with a distance of 30 cm between their midribs (Fig. 1) at a distance of 0.9 m from the bank and at least 1.5 m from the nearest plants or neighbouring test system. Generally two different experiments were run simultaneously, occasionally three.

Dead damselflies were used as models which were captured and prepared just before the experiments. After about 3 h body colours began to become noticeably dull and darkened and for this reason were no longer used. Models were fixed to the leaves with insect pins. Females were placed in an egg-laying position, while males were fixed at the level of the prothorax of the female in

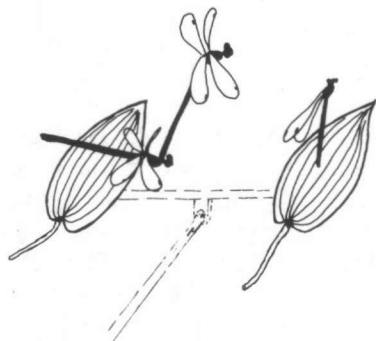


Fig. 1. Experimental setup for investigating the choice of oviposition site in *Coenagrion puella*. Two floating leaves of *Potamogeton natans* are fixed underwater to a cross piece attached to the end of a pole. The damselfly models, which are swapped around after each landing, are fixed to the leaves.

a vertical position. In the following experiments the number, position and arrangement of models were changed. In addition the models were reduced.

When a tandem landed at one of the prepared substrates the direction of approach, landing site, duration of stay and behaviour of the pair were recorded. If a female touched the plant material with her abdomen this behaviour was considered as an attempt to oviposition. After the tandem flew off the leaves were taken out of the water in order to exchange the side of the model or parts of it, respectively. All experiments were statistical tested for the homogeneity of the two observed distributions.

Unmated damselfly males temporarily attacked the pinned models. The object of these attacks was always the tandem-male. They landed on his body, their claspers gripping the prothorax, and tried to fly away with him. Tandem-females were not attacked. This behaviour was often observed at the beginning of the daily oviposition period. A detailed evaluation of the attacks will not be presented here.

Plant material was used that contained no eggs from previous ovipositions. The floating leaves of *P. natans* were taken from plants, which were excluded from oviposition by cages. The plant material was attached to sticks as described in the discrimination experiments and a dead male was pinned on it in vertical position. Egg deposition rate was calculated by dividing the total number of eggs found in the plant material by the observed duration of ovipositor contact. Only ovipositions of five and more eggs were evaluated. Video recordings of oviposition behaviour, a total of 22 situations, were used to check the field observations. A detailed description of the methods of investigating oviposition was given in a previous paper (MARTENS, 1992a).

RESULTS

CHOICE OF OVIPOSITION SITE

During choice experiments numerous tandems, generally in groups, were seen to occupy the ditch areas covered with *Potamogeton natans*. From here, only seldom directly from the bank, tandems appeared at the prepared sites of paired *P. natans* leaves. On 530 occasions (92.7% of all landings) the female curved her abdomen immediately on landing and touched the leaf with her ovipositor.

The presence of an ovipositing tandem attracted conspecific tandems searching for a breeding site (Tab. I). Tandems preferred groups rather than single tandems (Tab. II). There was no evidence for the attractiveness of a single female in egg-laying position. With a model of a single female in the course of 12 intervals of 20 min 8 landings nearby were recorded (Tab. I). Intermittently, in order to test for oviposition activity, the single female was replaced with the model of a tandem, whereby on average after 3.77 minutes a landing was registered. Over the same period of 4 h, during which 8 landing were registered near the single female, this would correspond to 64 landings.

A tandem with a male in the vertical position proved to be considerable more attractive than a tandem with a male in horizontal position (Tab. II), which, nevertheless was preferred to just a single horizontal male (14/1 landings; $\chi^2 = 11.3$; $P < 0.001$). The typical egg laying position of the females

had no noticeable effect (Tab. II). An upright male on its own, without a female, was attractive, too (Tab. I), although in direct comparison the number of landings was smaller (Tab. III). The body of a vertical male, without head, wings and legs, was also effective. A test series with a single horizontally perching male was stopped after 80 minutes when three different tandems had appeared at the occupied leaf. During the same period at two parallel sites with other models, 18 landings were recorded respectively.

Table I

Choice of oviposition site by tandems of *Coenagrion puella*.
Models of conspecifics are fixed to just one of the two leaves of *Potamogeton natans*.

Model	Landings on substratum		χ^2	P
	with model	without model		
Ovipositing tandem, male vertical	15	0	15.0	< 0.001
Ovipositing tandem, male sitting	15	1	12.3	< 0.001
Ovipositing female	8	3	2.3	NS
Vertical male	26	0	26.0	< 0.001
Vertical male, without head, wings and legs (torso)	14	1	11.3	< 0.001

Table II

Choice of oviposition site by tandems of *Coenagrion puella*.
Choice between a model of one ovipositing tandem with vertical male and other natural arrangements.

Other model	Landings on leaf with		χ^2	P
	the other model	ovipositing tandem, male vertical		
Group, two ovipositing tandems	26	6	12.5	< 0.001
Tandem, female with abdomen straight, male vertical	5	7	0.3	NS
Ovipositing tandem, male horizontal	9	27	9.0	< 0.01
Ovipositing female	1	16	13.2	< 0.001

Table III

Choice of oviposition site by tandems of *Coenagrion puella*.
Various reduced models were tested.

Model 1	Model 2	Landings on leaf with		χ^2	P
		model 1	model 2		
Tandem	vertical male	24	12	4.0	< 0.05
Male	Male, torso	18	7	4.8	< 0.05

Leaves carrying models of other damselfly species were visited when the bodies were pale blue marked with black (Tab. IV). In direct comparison tandems preferred the proximity of conspecific models, but *C. puella* did not differentiate between conspecifics and *C. pulchellum* (Tab. V) or *Enallagma cyathigerum* (2 versus 2 landings).

Table IV

Choice of oviposition site by tandems of *Coenagrion puella*.
Test of interspecific attraction. In each case the model was a vertical male.

Model	Landings on leaf		χ^2	P
	with model	without model		
<i>Platynemesis pennipes</i>	12	0	12.0	< 0.001
<i>Coenagrion pulchellum</i>	16	1	13.2	< 0.001
<i>Pyrrosoma nymphula</i>	1	3		

Table V

Choice of oviposition site by tandems of *Coenagrion puella*.
Test of discrimination between conspecifics and heterospecifics.
In each case the model was a vertical male.

Other male (Species)	Landings on leaf with		χ^2	P
	other male	<i>C. puella</i> -male		
<i>Coenagrion pulchellum</i>	21	27	0.75	NS
<i>Coenagrion hastulatum</i>	5	14	4.26	< 0.05
<i>Platynemesis pennipes</i>	14	39	11.79	< 0.001
<i>Erythromma najas</i>	4	24	14.29	< 0.001
<i>Pyrrosoma nymphula</i>	1	26	23.15	< 0.001

Females arrived directly at the petiole in 45% of initial landings ($n = 530$). Therefore a test was carried out to demonstrate that leaves with petioles recognizable to approaching tandems were preferred. A pair of floating leaves was used, both without petiole and carrying a model of a vertical male. Alternately a petiole was attached to one of the leaves, fixed below the water surface with an insect pin. Tandems showed a significant preference for the leaf with the attached petiole (25 versus 6 landings; $\chi^2 = 11.7$; $P < 0.001$), although only in 80% of the landings did the female actually land at the petiole.

OVIPOSITION ON *POTAMOGETON NATANS*

The contact of the female's abdomen with the plant material lasted between 2 s and 12.47 min ($n = 113$). Not every visit with oviposition behaviour resulted in eggs being laid. Distinguishing between actual oviposition and unsuccessful oviposition by mere observation was impossible in the field because insertion movements were not clearly recognizable. Eggs were placed almost exclusively

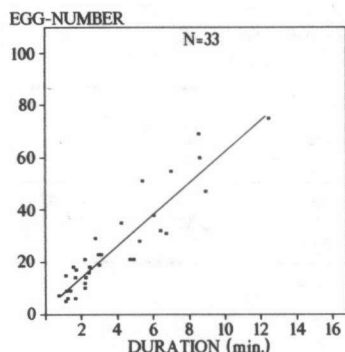


Fig. 2. Number of eggs of *Coenagrion puella* deposited into petioles of *Potamogeton natans* in relation to length of time the ovipositor was in contact with substrate. Data are only for ovipositions of 5 and more eggs ($n = 33$; $f(x) = 6.03x + 2.36$; $r = 0.92$, s.d. = 7.20; $P < 0.01$).

into the petiole and the pulpos leaf basis (Fig. 2). With the female moving slowly backwards and submerging her abdomen in the water a petiole was used along its whole length. Undisturbed ovipositions lasted 3.72 ± 2.47 min s.d. ($n = 28$) and 25.2 ± 17.3 s.d. eggs ($n = 28$) were laid. Eggs were deposited at a mean rate of 7.07 eggs/min (± 2.25 eggs/min s.d.; $n = 33$; range = 4.26 – 12.82 eggs/min). Egg deposition rate was not correlated to water temperature ($r_s = -0.0196$; NS; water temperatures between 17.2 and 22.8°C; $n = 33$) nor to air temperature in the shade ($r_s = -1.933$; NS; range 19.1 – 22.7°C; $n = 33$). Short stays without actual oviposition at the petiole had a mean duration of 16 s (± 12 s s.d.; $n = 8$; maximum = 40 s), contact to the underside of the leaf blade without oviposition 10 s (± 9 s s.d.; $n = 17$; maximum = 34 s).

On three occasions some eggs were inserted in the region of the mid rip (16 s (2 eggs), 23 s (3 eggs), 40 s (2 eggs)). The top side of leaves were palpated on average for 8 s (± 5 s s.d.; $n = 8$), and no eggs were deposited.

DISCUSSION

AGGREGATION BEHAVIOUR AND OVIPOSITION SITE SELECTION

During postcopula tandems of *Coenagrion puella* perform aggregation behaviour, similar to *C. pulchellum* and *Platycnemis pennipes* (MARTENS, 1989, 1992b). Groups develop because tandems prefer to land at sites where ovipositing pairs are already present. Tandems with vertical male are particularly attractive (Fig. 3). The guarding male may be considered to provide the actual stimulus. Comparable series of experiments have already been carried out with *P. pennipes*, which is very similar to *C. puella* in colour, size and tandem position of the male (MARTENS, 1992b). The experimental results were very similar, there being just two noticeable differences :

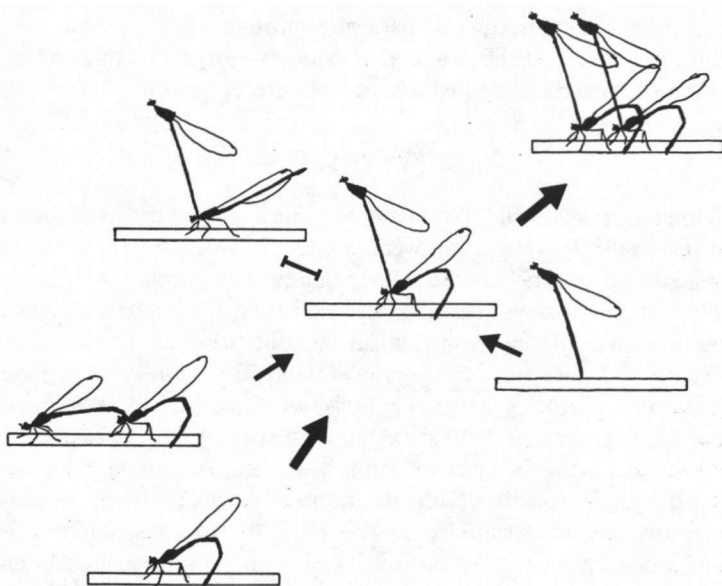


Fig. 3. A schematic representation of the results of the experiments on the choice of oviposition site in *Coenagrion puella*. Thick arrows represent a clear preference ($P < 0.001$) between two alternative sites, while thin arrows represent a recognizable preference ($P < 0.05$); a bar indicates that no preference was discernable.

(1) Given the choice between two alternative models, *C. puella* discriminates against the reduced form more clearly than *P. pennipes*.

(2) While *P. pennipes* does not differentiate between conspecifics and the likewise light-blue and black individuals of *Coenagrion*, *C. puella* clearly distinguishes its own species from *P. pennipes*, but not from *C. pulchellum*, the male of which is clearly more darkly coloured.

For oviposition *C. puella* landed on structures floating on the water or projecting from the water surface. The choice of oviposition site is influenced by the presence of conspecifics and by the presence of certain plant structures. With *Potamogeton natans* eggs are placed almost exclusively in the petiole. I had the impression that tandems preferentially choose to land at the petiole. Experiments in which a choice was offered showed that it is in fact the case, that tandems preferentially land at the base of the petiole, even when it is submerged. But this is not always the case. Often tandems behave opportunistically, landing on the blade of the leaf even though the finely structured petiole itself cannot be recognized. The female then searches for the petiole for a certain time with her abdomen. If she is unsuccessful, after briefly flying up, she searches another area of the leaf until she eventually

finds the petiole. This behaviour pattern is probably of particular use where vegetation is dense and the number of petioles is high. In this way suitable oviposition sites can be found which are not detectable in flight.

OVIPOSITION

A female *C. puella* will lay virtually the ripe eggs that are available (BANKS & THOMPSON, 1987). When eggs were laid the previous day the mean number of deposited eggs was 191 on the following day. The number of eggs increases with the number of days passed since the last oviposition took place. The maximum number of eggs to be laid in one day was found to be about 400, with a space of 5 days since the previous oviposition (THOMPSON, 1989). The average number of ripe eggs in *C. puella* is not stated by BANKS & THOMPSON (1987) or by THOMPSON (1989) since the frequency of oviposition depends on the weather and is thus subject to strong variation. Assuming the production of just 191 eggs/day, considering the average number of eggs deposited at each oviposition as determined in this study of 25 eggs/batch, it is clear that a pair of *C. puella* must deposit its eggs at a number of different sites. The average period in tandem is about 86 minutes (BANKS & THOMPSON, 1985), although the amount of this time spent in actually placing eggs is relatively small: for the deposition of 191 eggs at a rate of 7 eggs/min as found in this study a tandem would need only 27.3 minutes. As BANKS & THOMPSON (1985) have pointed out, a considerable amount of the remaining time is spent in searching for suitable oviposition sites.

C. puella prefers to deposit its eggs in vegetable parts which are wet or submerged. The waxy upper leaf surface and other unwetted parts are avoided. Many of the observations of oviposition and of plants used for oviposition in the literature need to be checked out. One should only speak of oviposition when eggs are actually shown to have been deposited.

THE UPRIGHT MALE GUARDING POSITION

The striking vertical guarding position of male damselflies has been known for a long time, being described by WESENBERG-LUND (1913) as the "*Agrion*"-type. Even today the significance of this posture is still poorly understood:

(1) The posture reduces the risk of predation. When attacked by green frogs *C. puella* tandems are better able to escape when the male is in an upright position. This enables them to remain longer within range of an approaching predator (REHFELDT, 1991).

(2) The posture stimulates on the formation of aggregations of ovipositing tandems. A vertical tandem-male has a much stronger attractive effect on other tandems than does a horizontal one (Table II). The male's vertically positioned

body provides the decisive visual cue (Table I). Oviposition in groups has many effects of its own which cannot be discussed here.

Other hypotheses are also conceivable :

(3) The male's posture makes it possible for the tandem to land on smaller pieces of substrate than would otherwise be the case, since he requires no space for himself. The tops of otherwise submerged plants can be landed on. The range of potential oviposition sites is decisively increased.

(4) It is possible that the posture prevent overheating of the male's body. Perching Anisoptera assume a vertical posture with their abdomen, the so called obelisk posture, particularly when it is very hot. In this way the sun is presented with the smallest possible surface area (e.g. MAY, 1977). Why should this not also be the explanation for the vertical posture of the tandem-male in damselflies ? Since this would make sense for the males of many species, particularly those which carry out oviposition in the heat of the midday sun. Females, on the other hand, have the possibility of losing heat through the direct contact of their abdomen with the water or through evaporation from their wetted bodies.

(5) The vertical posture may offer better protection through shielding the female's prothorax from rival males trying to split the tandem. In the horizontal posture the size of the unprotected angle between male and female dorsa is increased, thus offering a larger area of female prothorax to the claspers of an attacking rival male.

Which of the suggested possibilities are of particular importance cannot be determined. Certainly not all of the possibilities will be realized in a single species. For example, in *C. puella*, while aggressive attacks on tandems have been observed, this has never led to tandem splitting during oviposition (BANKS & THOMPSON, 1985 ; REHFELDT, 1991). In this species the vertical posture of the male may not play a role in providing protection from rivals.

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