

**INFLUENCE OF CONSPECIFICS AND PLANT STRUCTURES
ON OVIPOSITION SITE SELECTION IN
PYRRHOSOMA NYMPHULA (SULZER)
(ZYGOPTERA: COENAGRIONIDAE)**

A. MARTENS

Zoologisches Institut der Technischen Universität Braunschweig,
Pockelsstrasse 10a, D-38106 Braunschweig, Germany

Received January 31, 1993 / Revised and Accepted April 6, 1993

Tandems aggregate during oviposition. Discrimination experiments show that a motionless ♂, fixed to a floating leaf of *Potamogeton natans* in the typical vertical position of a tandem ♂, attracts conspecific pairs to land and oviposit at the same site. Successful oviposition occurs only within distinct plant parts, in the case of *P. natans* almost exclusively into leaf base and petiole, where tandems most frequently land. In the choice of oviposition site, sparkling reflections from the uneven water surface, caused by the edges of water plants, seem to play an important role. On *P. natans* petioles actual oviposition lasts on average 9.9 min, with a mean egg deposition rate of 5.56 eggs/min. During short stays of less than one minute no actual oviposition normally takes place.

INTRODUCTION

In dragonflies, vision plays an important role in discovering oviposition sites (WILDERMUTH, 1992). Species which deposit eggs into plant tissue search for oviposition sites in flight, and oviposition occurs only while settled. The choice of the landing site ("initial preference"; WAAGE, 1987) is the first step towards the possible utilization of a substrate.

Tandems of *Pyrrhosoma nymphula* often oviposit in very close proximity to one another. In this species REHFELDT (1990) demonstrated that aggregations of pairs provide protection against green frogs. Frogs prey upon solitary tandems and groups with similar success, but the predation risk to an individual tandem in a group is reduced. Further Rehfeldt was able to show that pairs already present influence approaching tandems in their choice of landing locations.

Do groups of ovipositing pairs develop because approaching tandems actually prefer to land close to conspecific pairs? Experimental proof of aggregation behaviour has already been obtained for tandems of *Coenagrion pulchellum*, *C. puella* and *Platynemis pennipes* (MARTENS, 1989, 1992b, 1993) as well as for females of *Calopteryx maculata* (WAAGE, 1987) and *Platycypha caligata* (MARTENS & REHFELDT, 1989). In this paper I try to provide similar proof of aggregation for *P. nymphula*, using experiments with models.

In addition, in the study of aggregation behaviour I will consider the influence of structural features of water plants on oviposition site selection.

MATERIAL AND METHODS

Pyrhosoma nymphula is a medium-sized damselfly, widely distributed throughout Europe. The abdomen of the male is red. The flight period ranges from April to August (ASKEW, 1988). Mate finding and copulation take place in the bank vegetation (REHFELDT, 1990). Eggs are laid into various floating and emergent water plants. Oviposition into *Potamogeton natans* has been described frequently (e.g. MACAN, 1964; RAU, 1966; STARK, 1977).

Observations were performed 15 km E of Braunschweig from 6 to 17 May, 1990, on 30 and 31 May, 1991 and from 14 to 23 May, 1992, at a ditch surrounding a man-made pond (Germany: 52°18'20"N, 10°46'20"E). The southern part of the ditch has steep banks densely covered with *Juncus effusus* and *Scirpus sylvatica*, the water was covered with *Potamogeton natans*. Discrimination experiments were carried out on a 10 m long section without water plants.

In order to evidence the occurrence of aggregation behaviour, two fresh floating leaves of *P. natans*, which were chosen to be as similar as possible, were attached to a submerged frame with metal clips. The leaves were cut off with their petioles and fixed parallel, at a midrib distance of 30 cm. They were placed 0.9 m from the bank and at least 1.5 m from the nearest plants or a neighbouring experimental set up. Damselflies, captured and prepared just before the experiments, were used as models. A tandem was fixed to one of the leaves with insect pins, the female with curved abdomen

and the male in a vertical position. Later the model was reduced to the vertical male (MARTENS, 1993, fig.). 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. Additionally, 33 recordings were taken with a video camera. If a female touched the plant material with her abdomen, this behaviour was considered as an attempt to oviposit. After the tandem flew off, the leaves were taken from the water and the model was fixed to the other leaf.

To determine the influence of plant structure on oviposition site selection, the experimental design was varied in different ways. At first I continued to use pairs of *P. natans* leaves, now

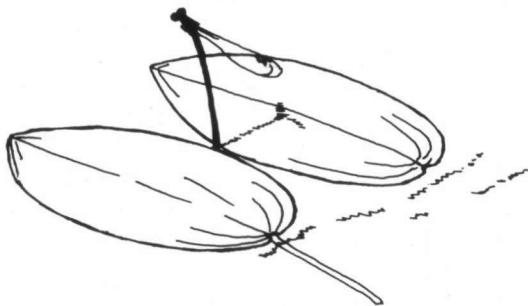


Fig. 1. An experimental setup for testing the influence of plant structures on oviposition site selection in *P. nymphula*. Two floating leaves of *Potamogeton natans* are fixed to a frame under water. To one of the leaves the petiole is refixed with an insect pin. A dead male is pinned between the two leaves.

placed directly next to each other. A dead male was fixed in a vertical position between both leaves. In order to compare the attractiveness of leaf apex and leaf base, the leaves were arranged apex to base and reversed after each landing. In order to examine the role recognition of the petiole plays in landing behaviour, the petioles of both leaves were cut off at the leaf base, and one was alternately attached to one of the leaves, fixed below the water surface with an insect pin (Fig. 1).

In order to test the influence of the leaf edge, an artificial circular leaf form was chosen. Discs of 45 mm diameter were punched out of leaves of *Nuphar lutea* with a tin and divided into four sectors of equal size. A vertical male was placed in the centre of each disc. The smooth edge was provided with a semicircular indentation (radius 2.5 mm), using a punch on two of the four sectors. In this way a circular reflection, easily discernible for the human eye, was made at the edges of two sectors, while it was lacking on the other two sectors. After each landing the leaf was revolved 180°, while the vertical damselfly model retained the same direction as before.

Oviposition was investigated on floating leaves of *P. natans*. The leaves were taken from plants in the ditch, which had been protected against oviposition from damselflies by cages since 1 May. Oviposition was timed from initial contact of the female's abdomen with the plant material until the last contact. Water temperature, 2 cm below the water surface, and air temperature in the shade were measured at the same time. The leaves were collected and kept for a maximum period of 14 days at 16°C. Afterwards eggs were counted under a dissecting microscope and the plant material was conserved in 70% ethanol. A detailed description of the method of investigating oviposition of damselflies has been given in a previous paper (MARTENS, 1992a). Egg deposition rate was calculated by dividing the total number of eggs found in the plant material by the observed duration of ovipositor contact.

RESULTS

CHOICE OF OVIPOSITION SITE

During choice experiments numerous tandems were seen at the ditch areas covered with *Potamogeton natans*. They changed their oviposition sites at irregular intervals, and generally they formed small groups. Single tandems left this area and flew parallel to the bank at a height of 10-15 cm over the water. As a rule they approached the prepared sites, sometimes coming directly from the bank. Approaching tandems showed a significant preference for the leaves carrying the conspecific models (Tab. I). The female curved her abdomen immediately after landing and touched the leaf with the ovipositor. For floating leaves of *P. natans*, the leaf base was the preferred substrate (Tab. II), and it made no difference whether or not the leaf was provided with a dragonfly model ($\chi^2 =$

Table I

Choice of oviposition sites in *P. nymphula*. Models of conspecifics are alternately fixed to one of two *Potamogeton natans* leaves

Model	Landings on leaf		Difference from 1 : 1	
	with model	without model	χ^2	P
Ovipositing tandem	15	6	3.86*	< 0.05
Vertical male	28	7	12.60	< 0.001

1.07; $df = 3$; NS). The direct experimental comparison of choice between leaf apex and base confirmed that the leaf base was the preferred substrate (Tab. III). Tandems landed on the leaf base indiscriminately whether or not the petiole was absent (Tab. III). On the artificial, circular leaves tandems preferentially landed on sectors which contained the small indentation in their edge (Tab. IV).

OVIPOSITION

Contact of the female's abdomen with the leaf lasted between 3 s and 26.22 min ($n=102$). Not every visit with oviposition behaviour resulted in eggs being laid. Distinguishing between the actual oviposition and the unsuccessful oviposition by mere observation was impossible, since the insertion movements were not clearly recognizable. The examination of the collected leaves showed that eggs were not deposited during short stays when the ovipositor was in contact with the leaf for less than one minute (Tabs V, VI), with a single exception: 31 s for one egg. The actual undisturbed oviposition lasted on average 9.90 min (± 6.56 min s.d.; $n=22$).

Eggs were placed almost exclusively into the petiole and the fleshy leaf base. The petiole could be used along its whole length when the female moved slowly

Table II

Place of first ovipositor contact of *P. nymphula* on leaves of *Potamogeton natans* during aggregation experiments (Tab. I)

Substrate	Frequency of landings at leaves			
	with model		without model	
	%	(n)	%	(n)
Upper leaf surface	4.7	(2)	0.0	(-)
Underside, lateral	30.2	(13)	38.5	(5)
Underside of apex	11.6	(5)	7.7	(1)
Petiole / leaf base	53.5	(23)	53.8	(7)
<i>Total</i>		(43)		(13)

Table III

Choice of oviposition sites in *P. nymphula*. Test of the attractiveness of various leaf parts of *Potamogeton natans*. Only the choice of direct alternatives of adjacent parts of two neighbouring leaves are taken into account

Presented alternatives		Landings on		Difference from 1:1	
A	versus B	A	B	χ^2	P
Leaf base	apex of leaf	21	7	7.00	< 0.01
Leaf with petiole	leaf without petiole	16	17	0.03	NS

Table IV

Influence of leaf edge on oviposition site selection in *P. nymphula*. Circular pieces of *Nuphar lutea* leaves serve as substrate. Two sections of one piece have a smooth edge, while the other two sections each have a small indentation

Site	Approaches without landing	Landings with attempted oviposition
Leaf segment with smooth edge	12	3
Leaf segment with one indent	3	11

$\chi^2 = 9.95$; $df = 1$; $P < 0.01$

backwards and submerged her abdomen. During the undisturbed oviposition a female deposited a mean of 57.7 eggs per visit (± 43.4 s.d.; $n=22$). The leaf blade was found to contain a small number of eggs on only 6 occasions (1, 3, 4, 5, 9 and 14 eggs). They had been inserted in the region of the midrib. The dry top side of leaves were palpated without any egg deposition taking place (Tab. V).

The relationship between duration of oviposition and egg deposition rate is presented in Figure 2. Eggs were deposited at a

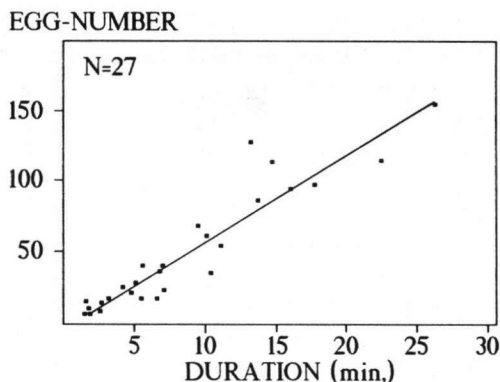


Fig. 2. Number of eggs laid by *P. nymphula* in stalks of *Potamogeton natans* in relation to the length of stay in the attitude of oviposition. — [Data for ovipositions of 5 and more eggs; — $n = 27$; — $f(x) = 6.13x - 3.07$; — $r = 0.94$; — $s = 15.06$; — $P < 0.01$].

Table V

Substrate utilization by *P. nymphula*; comparison of frequency of successful oviposition and maximum egg-number at different parts of *Potamogeton natans* leaves

Substrate	% successful ovipositions	Maximum egg-number	No. samples checked
Upper leaf surface	0	0	13
Underside, lateral	10	5	20
Underside of apex	12.2	14	31
Petiole / leaf base	76.5	155	34

mean rate of $5.56 \text{ eggs min}^{-1}$ (± 1.74 s.d.; $n=27$). Water temperatures ranged between 17.4 and 22.6°C , air temperatures in the shade between 19.0 and 24.8°C . Egg deposition rate was found to be more strongly correlated to water temperature ($r_s=0.592$; $P<0.01$; Fig. 3) than to air temperature ($r_s=0.440$; $P<0.05$).

Table VI

Duration of unsuccessful oviposition attempts by *P. nymphula* on different parts of *Potamogeton natans* leaves

Substrate	Duration (s)		(n)
	Median	Max	
Upper leaf surface	10	25	(13)
Underside, lateral	9	25	(18)
Underside of apex	14	56	(27)
Petiole / leaf base	13	51	(8)

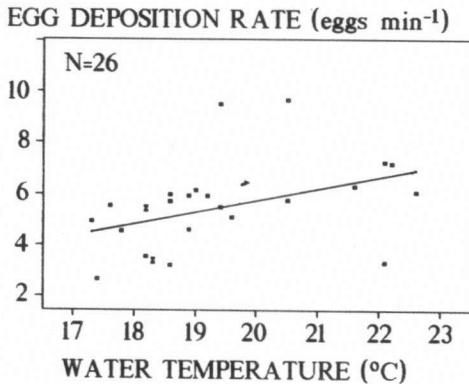


Fig. 3. Relation between water temperature and egg deposition rate for *P. nymphula*. — [Only ovipositions of 5 and more eggs per petiole of *Potamogeton natans* were evaluated; — $n = 26$; — $f(x) = 0.45x - 3.25$; — $r = 0.42$; — $s = 1.59$; — $P < 0.05$].

small oviposition site, therefore the presence of a complete model results in a considerable reduction of the available site area, causing an approaching tandem to fly on to the next available leaf.

The fact that a vertical male alone is a sufficient stimulus, as is also the case in *Platycnemis pennipes* and *Coenagrion puella* (MARTENS, 1992b, 1993), could be explained in terms of the oviposition behaviour. During oviposition the female is often partially or wholly submerged (MÜNCHBERG, 1935; ROBERT, 1958), so that she may be difficult to see. The male's vertical position is possible only when in tandem with a female.

For oviposition *Pyrhosoma nymphula* is able to use only a certain part, the petiole, of the floating *P. natans* leaf (Tab. V). Although the base is only a small part of the leaf, tandem pairs frequently land and the female can immediately apply her ovipositor to it (Tabs II, III). With respect to this particular plant, there is a great similarity in choice and utilization of oviposition sites by *Coenagrion puella* (MARTENS, 1993). However, in contrast to *C. puella*, recognition of the petiole is insignificant for approaching *P. nymphula* in the choice of oviposition site (Tab. III; MARTENS, 1993), whereas the leaf edge is of particular importance. In my opinion, the leaf edge plays an indirect rather than a direct role in the recognition of oviposition sites, the reflection or the refraction pattern from the water surface being the direct stimulus. The base of *P. natans* leaves contains a specific structure producing a small circle of reflexion at the water surface, which partly resembles the patterns produced at the leaf apex and also on the sides of undulated or damaged leaves. Here, even though less frequently, eggs are also

DISCUSSION

The presence of a motionless conspecific tandem on a suitable floating substrate is an important stimulus for attracting the approaching tandems to land at the same site. The male in its typical vertical tandem position is quite a sufficient stimulus without a female being present.

The fact that the experiments with a complete tandem model were less conclusive than those in which the model was reduced to just a vertical male may have its cause in the method used. A single leaf of *P. natans* is a relatively

deposited. Using an artificial leaf form, showing the relevant edge structure, it is possible to elicit oviposition behaviour (Tab. IV). However, since it has not been possible to manipulate this reflection pattern, a direct proof of this hypothesis is still lacking.

An important effect of glitter patterns of the water caused by vegetation on the habitat selection of a dragonfly has already been suggested by WILDERMUTH (1987). The experimental imitation of the reproductive habitat of *Soma-tochlora arctica* has clearly shown that stretched shining black plastic foil suffices to stimulate significant elements of reproductive behaviour in that species (WILDERMUTH & SPINNER, 1991).

For a *P. nymphula* population in northern England, GRIBBIN & THOMPSON (1990) give 351 ripe eggs as the mean per female. This value can only be considered as an approximation, which is dependent on sample date. In addition, in the said study the data obtained from unmated (and thus possibly infertile/non-receptive) females, from females in copula and from females already conducting oviposition are lumped together. Although there are possible differences in the fertility between N English and N German populations, and the number of deposited eggs per clutch varies with the plant species used, combining my data with those of Gribbin & Thompson gives a clear indication that a pair distributes its eggs between different sites during its daily oviposition activity. With an average of 58 deposited eggs per a *P. natans* petiole one can suggest that an individual tandem will distribute its eggs at six different locations. Assuming the mean egg deposition rate at 351 eggs/day and the deposition rate during the actual oviposition at 5.56 eggs/min, an individual tandem will need 63 min of actual oviposition time, not including the exploratory activity.

As in the case of *Platynemís pennípes* (MARTENS, 1992a), it has been found that only during periods of extensive ovipositor contact with the substrate one can assume that oviposition actually takes place. Both species prefer to lay eggs in the wet or submerged parts of the plant. The waxy top side of the leaf and other dry areas are avoided. Many of the observations on oviposition reported in the literature, therefore, need to be checked. In the case of endophytic deposition, one should only speak of oviposition substrates when eggs are actually found to have been laid.

ACKNOWLEDGEMENTS

My thanks to KARIN NEUBAUER and ADELHEID MARTENS for their support in the field. I am grateful to PETER MILLER, PETER SCHRIDDE, FRANK SUHLING and HANSRUEDI WILDERMUTH for helpful criticism on the manuscript. The Bezirksregierung Braunschweig gave me permission to catch specimens of *P. nymphula*.

REFERENCES

- ASKEW, R.R., 1988. *The dragonflies of Europe*. Harley Books, Martins.
- GRIBBIN, S.D. & D.J. THOMPSON, 1990. Egg size and clutch size in *Pyrrhosoma nymphula* (Sulzer) (Zygoptera: Coenagrionidae). *Odonatologica* 19: 347-357.
- MACAN, T.T., 1964. The Odonata of a moorland fishpond. *Int. Revue ges. Hydrobiol.* 49: 325-360.
- MARTENS, A., 1989. Aggregation of tandems in *Coenagrion pulchellum* (Vander Linden, 1825) during oviposition (Odonata: Coenagrionidae). *Zool. Anz.* 223: 124-128.
- MARTENS, A., 1992a. Egg deposition rates and duration of oviposition in *Platynemis pennipes* (Pallas) (Insecta: Odonata). *Hydrobiologia* 230: 63-70.
- MARTENS, A., 1992b. *Aggregationen von Platynemis pennipes (Pallas) während der Eiablage (Odonata: Platynemididae)*. Ph. D. Diss. Tech. Univ. Braunschweig.
- MARTENS, A., 1993. Field experiments on aggregation behaviour and oviposition in *Coenagrion puella* (L.) (Zygoptera: Coenagrionidae). *Adv. Odonatol.* 6. — [In press].
- MARTENS, A. & G. REHFELDT, 1989. Female aggregation in *Platycypha caligata* (Odonata: Chlorocyphidae): a tactic to evade male interference during oviposition. *Anim. Behav.* 38: 369-374.
- MÜNCHBERG, P., 1935. Ueber die Fortpflanzungsverhältnisse, insbesondere die Paarung und Eiablage der Zygoptera Nordostdeutschlands. (Ordnung: Odonata). *Abh. Ber. Naturw. Abt. grenzmärk. Ges. Erforsch. Pflege Heimat, Schneidemühl* 10: 121-131.
- RAU, U., 1966. Die Odonatenfauna des Naturschutzparkes Hoher Vogelsberg. *Dt. ent. Z.* (NF) 13: 393-446.
- REHFELDT, G.E., 1990. Anti-predator strategies in oviposition site selection of *Pyrrhosoma nymphula* (Zygoptera: Odonata). *Oecologia* 85: 233-237.
- ROBERT, P.-A., 1958. *Les libellules (odonates)*. Delachaux & Niestlé, Neuchâtel.
- STARK, W., 1977. Ein Teich in der Steiermark (Österreich) als Lebensraum für 40 mitteleuropäische Libellenarten. *Ent. Z., Frankf./Main* 87: 249-263.
- WAAGE, J.K., 1987. Choice and utilization of oviposition sites by female *Calopteryx maculata* (Odonata: Calopterygidae). I. Influence of site size and the presence of other females. *Behav. Ecol. Sociobiol.* 20: 439-446.
- WILDERMUTH, H., 1987. Fundorte und Entwicklungsstandorte von *Somatochlora arctica* (Zetterstedt) in der Schweiz (Odonata: Corduliidae). *Opusc. zool. flumin.* 11: 1-10.
- WILDERMUTH, H., 1992. Visual and tactile stimuli in choice of oviposition substrates by the dragonfly *Perithemis mooma* Kirby (Anisoptera: Libellulidae). *Odonatologica* 21: 309-321.
- WILDERMUTH, H. & W. SPINNER, 1991. Visual cues in oviposition site selection by *Somatochlora arctica* (Zetterstedt) (Anisoptera: Corduliidae). *Odonatologica* 20: 357-367.