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COMMUNICATION

BREEDING BEHAVIOUR OF THE COROMANDEL MARSH DART DAMSELFLY (ZYGOPTERA: COENAGRIONIDAE: *CERIAGRION COROMANDELIANUM* (FABRICIUS)) IN CENTRAL INDIA

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Breeding behaviour of the Coromandel Marsh Dart Damselfly (Zygoptera: Coenagrionidae: *Ceriagrion coromandelianum* (Fabricius)) in central India

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Abstract: *Ceriagrion coromandelianum* (Fabricius) is one of the most common damselflies in the Indian subcontinent. It flies among bushes and breeds in stagnant pools, small garden tanks, tubs and ornamental cement ponds containing submerged and/or floating vegetation. The oviposition behaviour of *C. coromandelianum* was observed at the botanical garden of Hislop College, Nagpur, India, where small underground cement tubs are utilized to grow macrophytes. *C. coromandelianum* displays a refined hierarchy of preferences for oviposition and chooses floating leaves of *Nymphaea nouchali* (69%) over *Lemna paucicostata* (23%) and submerged *Hydrilla verticillata* (8%). In an uninterrupted oviposition bout, the female deposits 283 eggs in 16 rows (N=5) on the under surface of the *N. nouchali* leaf. The tiny leaves of *L. paucicostata* holds 7.8 eggs in 4.8 rows (N=10). In *H. verticillata*, the internode region of the stem can house 25.4 eggs (N=10). One or two eggs are also found neatly inserted in the thin leaf base of *H. verticillata*. Decaying plant material is never used for oviposition. The present investigation also clearly demonstrates that the choice of oviposition substrate not only depends upon the presence of aquatic species in the water body but also on the spatial location of the oviposition site.

Keywords: Egg, endophytic, *Hydrilla verticillata, Lemna paucicostata, Nymphaea nouchali*, Nagpur, oviposition

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Author Contribution: NRT and PRV contributed in field work and documentation of the oviposition behaviour. RJA set up the project and evaluated the findings.

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INTRODUCTION

Habitat selection for oviposition is a vital and complex behavior undertaken by odonates, after the termination of copulation. Oviposition takes place either in water (aquatic), on floating plant material (epiphytic), or inside plant tissue (endophytic). For damselflies, choice of ovipositing material primarily depends upon "the initial preference" which is a suitable place both, for landing and easy deposition of egg in the plant tissue (Waage 1987; Martens 1992, 1993, 1994, 1996, 2001). Highly stenotopic species oviposit in one or very few species of plant while eurytopic species may exhibit distinct preferences and oviposit in a wide variety of plants (Martens 1996). Some odonate species of temperate regions show preferences in selecting plant species or even parts/region of plants for oviposition (Martens 1992; Wildermuth 1993; Grunert 1995).

Previously we found that the Coromandel Marsh Dart Damselfly *Ceriagrion coromandelianum* (Fabricius, 1798) selects specific laminar region of the leaf of water lily, *Nymphaea nouchali* f. for oviposition and also reported the existence of a direct co-relation between the choice of leaf lamina region and the day of oviposition (Andrew et al. 2011a). In this damselfly, we also studied and evaluated the process of contact guarding oviposition with reference to the male in sentinel position (Andrew et al. 2011b). In continuation with the study of the reproductive biology of this species, the present paper describes the oviposition of *Ceriagrion coromandelianum* with reference to choice, insertion and deposition of egg in the aquatic plants *Nymphaea nouchali* Burm, f., *Hydrilla verticillata* (L.f.) Royde and *Lemna paucicostata* Hegelm. 6746.

MATERIAL AND METHODS

Site: The observation and collection for the present work was carried out at the botanical garden of Hislop College, Nagpur, (21.16^{0}N & 79.03^{0}E) where small underground cement tubs/tanks are used to grow macrophytes (Image 1). There is a large circular tub (diameter 105cm) surrounded by six smaller circular tubs (diameter 42cm), followed by a row of three rectangular cement containers (84cm x 47cm). Height of the tubs varies between two to three feet. Two rectangular tubs contain floating *L. paucicostata* and submerged *H. verticillata* while the small circular tubs contain *H. verticillata.* The single large circular tub contains floating *N. nouchali,* small patches of *L. paucicostata* and submerged *H. verticillata* (Image 2). The bottom of the tubs comprise mud, debris and decaying leaves. *C. coromandelianum* is found ovipositing in these tanks all around the year (except from December to February). The plant material were collected after the female had completed egg deposition and taken to the laboratory for investigation under stereoscope binocular microscope.

RESULTS

Ceriagrion coromandelianum is one of the most common damselflies in the Indian subcontinent. The male is bright yellow with olivaceous and pale greenish yellow eyes. The females are initially as bright as the males but with maturity turn dull yellow to light brown. The intensity of dullness increases with maturity. It flies among bushes and breeds in stagnant pools and small garden tanks, tubs and ornamental cement ponds

Image 1. The study site: water tubs at Botanical garden of Hislop College, Nagpur.

Table 1. ***Ceriagrion coromandelianum*** **- number of ovipositing females on different plants over 20 days.**

Days	*N. nouchali*	*L. paucicostata*	*H. verticillata*
1	4	1	0
2	7	3	1
3	5	3	2
4	6	2	0
5	6	0	0
6	8	0	0
7	5	0	0
8	4	4	1
9	6	4	2
10	4	2	0
11	4	2	1
12	9	3	1
13	3	3	2
14	3	2	1
15	5	0	0
16	6	1	0
17	3	2	0
18	3	1	0
19	5	0	0
20	3	1	1
TOTAL	99	34	12
%	69	23	8
Mean	4.95	1.7	0.6
SD	2.06	1.41	0.75
SE	0.38	0.30	0.16

containing floating and/or submerged vegetation. Females visit the waters only for reproduction but the males can be spotted at all times around the shrubs near the ovipositing site.

For oviposition, the tandem pair of *C. coromandelianum* flies towards the tubs and alights on a floating substrate (*N. nouchali*, *H. verticillata, L. paucicostata*). The female starts probing the underside with the ovipositor located at the terminal tip of the abdomen. Initially, the female evaluates the under-surface of the substrate by feeling and testing the plant material with the stylus of her ovipositor. If she finds that the substrate is not suitable for oviposition, she repositions herself by moving a few inches along the rim of the leaf lamina (in case of *N. nouchali)* or relocates to a different site, not more than 5cm (in case of *H. verticillata* and *L. paucicostata*) away. Once settled, she begins to perforate the plant tissue with her saber shaped sharp ovipositor valvules and starts laying eggs

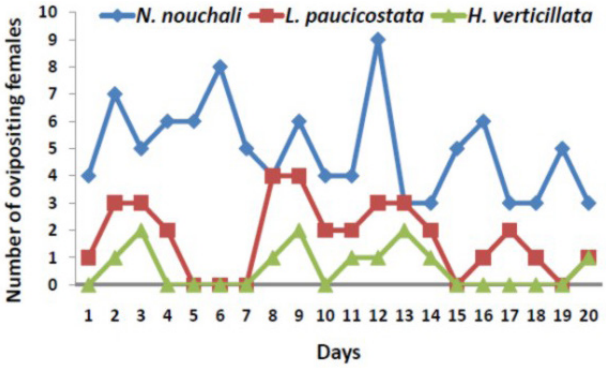

Figure 1. ***Ceriagrion coromandelianum*** **- graphical representation of oviposition on different plants.**

in it. The female never uses decaying plant material for oviposition.

Experiments were conducted by placing plastic leaf models of *Nymphaea* in a plastic water tub to understand the behaviour of oviposition. The tandem pair readily settled on the plastic leaf and the female started probing the under-surface of the artificial leaf with the ovipositor, but after hectic probing (168 Sec, Max-225; Min.- 15, N=5), the tandem pair left the site (Image 2). To understand the order of preference for oviposition substrate by *C. coromandelianum* the data of 20 days (when maximum number of oviposition was noticed) was evaluated. *C. coromandelianum* displays a refined hierarchy of preference for oviposition substrate and chooses floating leaves of *N. nouchali* (69%) over *L. paucicostata* (23%) and submerged *H. verticillata* (8%) (Table 1, Fig. 1).

Oviposition in *Nymphaea nouchali*

Leaves of *Nymphaea nouchali* form a perfect landing site for *C. coromandelianum* and provide a large surface area for oviposition (Image 3). In an uninterrupted oviposition bout, the female deposits 288 eggs (Max 322; Min 243; N=5) in 19 rows along the undersurface of the leaf (Image 3). The eggs are mostly arranged in slightly concentric rows and the distance between two eggs is about 689.4±26.5 µm (Max 1230µm; Min 230µm; N=100). The distance between two rows is 2.4±0.3 mm (Max 3.32mm; Min 1.6mm; N=5). Graphic representation by plotting the trend line of second order polynomial shows that maximum number of eggs are laid in the middle row (Table 2, Fig. 2).

Oviposition in *Lemna paucicostata*

The puncture mark of the ovipositor of *C. coromandelianum* can be easily observed on the

Table 2. ***Ceriagrion coromandelianum*** **- row wise number of eggs deposited in one bout of oviposition on the leaf of** ***Nymphaea nouchali.***

Row	Ovipositing female					
	1st	2nd	3rd	4th	5th	Total
1	10	14	7	8	7	46
2	11	27	16	10	12	76
3	15	25	25	22	26	113
4	18	42	14	13	15	102
5	9	38	34	11	14	106
6	17	19	7	10	7	60
7	11	30	16	16	15	88
8	13	8	25	17	18	81
9	18	10	14	33	34	109
10	13	24	34	34	38	143
11	14	7	10	7	43	81
12	17	12	42	14	23	108
13	15	15	26	12	21	89
14	21	6	18	39	-	84
15	17	-	12	26	-	55
16	11	-	7	18	-	36
17	7	-	4	19	-	30
18	6	-	8	17	-	31
19	-	-	3	-	-	3
Total	243	277	322	326	273	1441
%	16.9	19.3	22.3	22.6	18.9	100
SD	4.12	11.5	11.18	9.34	11.45	35.46
SE	0.97	2.71	2.63	2.20	2.70	3.36

reddish-brown undersurface of *L. paucicostata* (Image 4). Carefully peeling the leaf under the microscope reveals that the tiny leaf of *L. paucicostata* holds 37.8 eggs in 4.8 rows (Max 47; Min 30; N=10) (Table 3). The distance between two eggs is 182±20.6 µm and the distance between the two rows is 1610±70 µm (Max 1800µm; Min 1610µm; N=20). The statistical analysis of the data by plotting the trend line of second order polynomial reveals that the maximum numbers of eggs are laid in the middle rows (Fig. 3).

Oviposition in *Hydrilla verticillata*

In *H. verticillata*, the internode region of the stem is used for oviposition. Each internode region can house 25.4 eggs of *C. coromandelianum* (Max 33; Min 14; N=10) (Table 4). The eggs are arranged mostly in two to four longitudinal rows. There is a gap of 71.6±12.7 µm between the eggs. The rows are at a distance of 847.6±52 µm (Max 980µm; Min 670µm). Sometimes oblique or overlapping rows of eggs are also found in

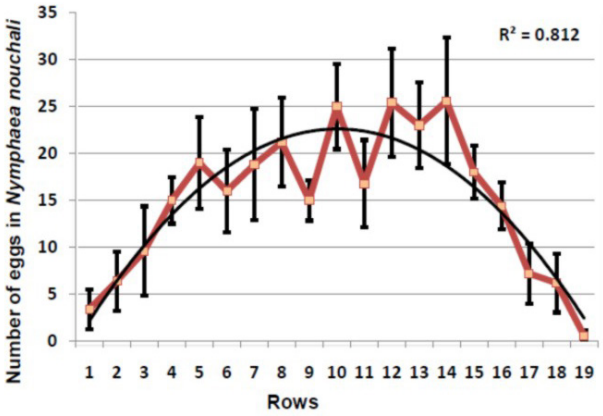

Figure 2. ***Ceriagrion coromandelianum*** **- the average number of eggs per row laid in the leaf lamina of** ***Nymphaea nouchali.***

Table 3. Number of eggs deposited by ***Ceriagrion coromandelianum*** **in the leaves of** ***Lemna paucicostata.***

Leaf	Row						Total	%
	1	2	3	4	5	6		
1	3	6	7	7	4	3	30	08
2	9	7	11	7	2	-	36	9.5
3	3	3	11	10	7	-	34	09
4	5	8	14	13	7	-	47	12.4
5	3	9	7	13	9	-	41	10.8
6	4	5	8	8	11	-	36	9.5
7	3	5	12	13	7	-	40	10.6
8	8	11	12	7	-	-	38	10
9	7	8	12	9	-	-	36	9.5
10	8	14	6	11	-	-	39	10.3
Total							377	100

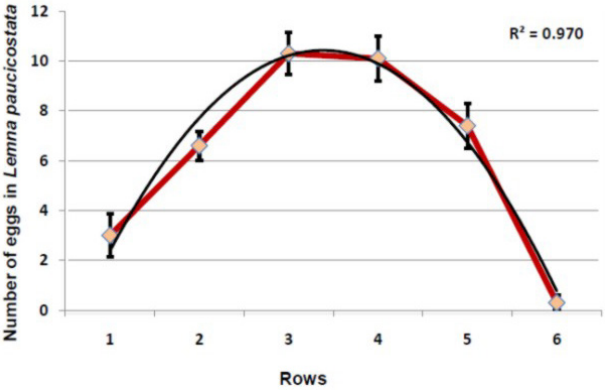

Figure 3. ***Ceriagrion coromandelianum*** **- the average number of eggs per row laid in the leaf lamina of** ***Lemna paucicostata.***

Table. 4. Number of eggs deposited by *Ceriagrion coromandelianum* in the internode of *Hydrilla verticillata*.

Sno	1	2	3	4	5	6	7	8	9	10	Total	Mean	SD	SE
Number of eggs	14	32	17	29	27	30	33	18	24	30	254	25.4	6.8	2.3

Image 2. *Ceriagrion coromandelianum* trying to settle and oviposit in artificial plastic leaf of *Nymphaea*. The experimental tub (a). Tandem pair landing on the leaf (b) and female trying to insert eggs along the under-surface of the plastic leaf (c).

Image 3. *Ceriagrion coromandelianum* ovipositing on leaf of *Nymphaea nouchali* (a,b). Rows of eggs inserted in the leaves (c) (photographed under dissecting microscope).

the internode region. Rarely, one or two eggs are found neatly inserted in the very thin leaf base of the plant (Image 5).

At times, the female sitting on *N. nouchali* dips the abdomen in water and the ovipositor encounters the underlying *H. verticillata.* She ensures the suitability of *H. verticillata* plant by probing it with the ovipositor and starts depositing eggs in it. This has been tested by deliberately placing or removing *H. verticillata* below *N. nouchali* during oviposition. If *H. verticillata* is removed, the female explores the surrounding areas with the ovipositor without moving from her position and begins ovipositing in *N. nouchali*, and, if *H. verticillata* is placed between the ovipositor and *N. nouchali* leaf, the female easily switches and starts ovipositing in *H. verticillata.*

After a complete duration of oviposition, the female dislodges herself from the tandem and flies away into the surrounding shrubs or darts away up to a different site. At times, the tandem couple exhibits the ritual of 'water-touching' after completing oviposition, i.e., the pair in tandem flies over the water body, and then the female dips her abdominal tip in the water and then the pair flies away among the surrounding bushes. The male is reluctant to leave the female and tries to either re-copulate or force her to re-oviposit by leading her back to water. Sometimes, the female releases herself from the male after resting in tandem for 2–7 minutes. The released female is at times followed by other males but she quickly darts away from the ovipositing site.

Image 4. ***Ceriagrion coromandelianum*** **ovipositing in** ***Lemna paucicostata*** **(a). Puncture oviposition marks on the reddish-brown under-surface of the leaf (b). Eggs placed in tiers in the leaf lamina (c)**

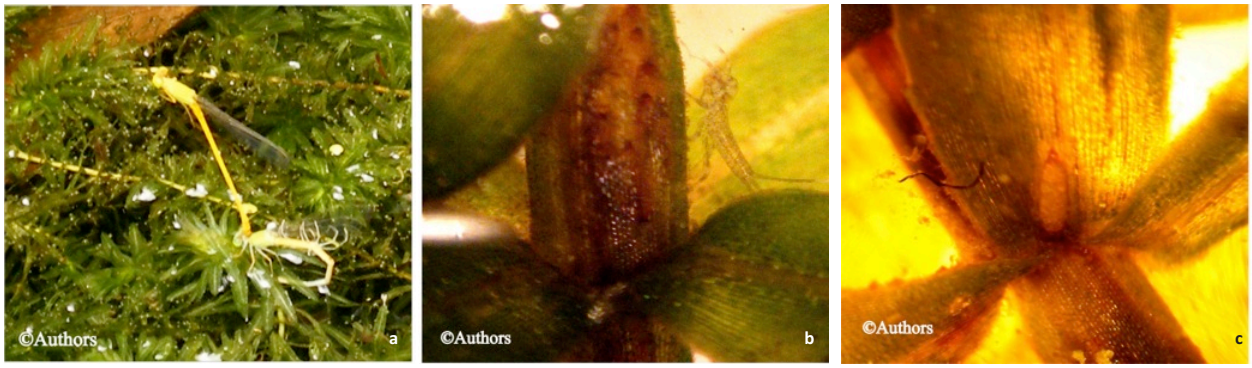

Image 5. ***Ceriagrion coromandelianum*** **ovipositing in** ***Hydrilla verticillata*** **(a) Oviposited eggs in the internode region of the stem of** ***H. verticillata*** **(b) (the apical brown region of the egg can be clearly demarcated while a semi transparent first instar larva clings to the plant). Single egg neatly placed in the leaf base (c).**

DISCUSSION

In *Ceriagrion coromandelianum*, the selection of the landing site for oviposition is visual. This was demonstrated by the experiment of using plastic models of *Nymphaea* leaves as site for oviposition, where the female readily alighted on the artificial leaf and started to feel the under-surface of this leaf for oviposition. Since the plastic was harder (than aquatic vegetation) the female could not penetrate the ovipositor and lay eggs. Once the female dips the abdomen inside water, it is the ovipositor which determines the choice of the insertion site, which can either be the landing substrate (*N. nouchali*), or the underlying *H. verticillata* as demonstrated in the experiment of removing or placing *H. verticillata* below the landing substance.

Matushkina & Gorb (2007) found that coenagrionids and platycnemidid damselflies predominantly choose tissues of Nymphaeaceae for oviposition, while lestids mostly laid eggs in coastal plants. *Lestes temporalis* and *Chalcolestes* spp. oviposit into woody branches of trees and bushes. *Aeshna viridis* used leaves of *Stratiotes aloides* while *Coenagrion mercuriale*, mostly used the aquatic *Berula erecta* (Matushkina & Gorb 2002). Matushkina & Lambret (2011) observed that before inserting her ovipositor in the plant, the damselfly *Lestes macrostigma*, touches the plant tissue six times before ovipositing. These touches may provide information leading to a choice of insertion site. The damselfly *Platycnemis pennipes* oviposit in at least 25 species of plants but while ovipositing into the flowering stem of *Nuphar lutea,* it also exhibits similar hierarchy of preferences in which the age of stems, colour, size and association with a floating leaf are taken into consideration (Martens 1996; Corbet 1999).

In India, Srivastava & Babu (1985) found that *C. coromandelianum* prefers the aquatic plant *Salvinia* over *Azolla* and never oviposits in *Spirodela polyrhiza, Trapa natans, Eichhornia crassipes, Lemna microphylla* and *Nelumbo* sp. or dead or decaying leaves at Sagar, Madhya Pradesh. Prasad (1990) found that it oviposits in all aquatic plants at Kolkata, West Bengal while

Sharma (2009) reported that it prefers *H. verticillata* and *Polygonum barbatum* over other aquatic plants at Hoshiarpur, Punjab. In the present study we have noticeably demonstrated the *C. coromandelianum* displays a refined hierarchy of preferences for oviposition and chooses floating leaves of *N. nouchali* over *L. paucicostata* and submerged *H. verticillata.* The present investigation clearly demonstrates that the choice of oviposition substrate not only depends upon the presence of aquatic species in the water body but also on the spatial location of the oviposition site.

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