Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com
JEZS Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(3): 35-39

Received: 05-03-2016
Accepted: 07-04-2016

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Association of larval Odonata and hydrophytes in wetlands of West Bengal, India: implications for conservation and monitoring

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Abstract
The abundance and distribution of the larval odonates (dragonfly and damselfly) depend on the hydrophytes, which was tested in the present study. A total of 19 genera of Odonata were observed in different relative abundance against the hydrophytes. Among the damselflies and dragonflies genera, *Ceriagrion* and *Pantala* respectively, remained dominant over others with significant variations in relative abundance in the samples. Hydrophytes like *Wolffia* and *Marsilea* were key factors in explaining the relative abundance of larval odonates as explained through the canonical correspondence analysis. The species specific abundance seemed highly dependent on the hydrophytes in the concerned water bodies. In order to enhance the sustenance of the Odonata in wetland habitats, availability of different species of hydrophytes seems to be an essential criterion. Considering the multifunctional role of the larval odonates in the freshwater aquatic communities, conservation effort should include the systematic inclusion of the preferred hydrophytes in the wetlands.

Keywords: Odonata, Canonical correspondence analysis, hydrophytes, wetland conservation

1. Introduction
Wetlands are characteristic larval habitat of different insect groups adapted to completion of the life cycle either in part or full in aquatic or semi aquatic conditions. Insects of the order Odonata, commonly known as dragonfly (suborder Anisoptera) and damselfly (suborder Zygoptera), spend larval stages in aquatic conditions. In aquatic habitats, odonate larvae exploit hydrophytes as refuge with other controphic and heterotrophic species. Association of odonate larvae with hydrophytes ensures availability of prey and defense against predators [1]. Odonate larvae are predators of varied organisms including oligochaetes, aquatic snails, crustaceans, insects, small fishes and tadpoles [2-5]. The generalist predatory nature of odonate larvae reflects their potential to regulate wetland species diversity. Empirical studies suggest that predatory activity of odonate larvae vary with the presence of hydrophytes [4]. While hydrophytes appear to facilitate odonate community assemblages of wetlands, few studies have quantified odonate species association with hydrophytes [6, 7]. In view of hydrophytes-odonate species interaction, the present study explores hydrophytes as predictors of larval odonate assemblages.
The odonate larvae are common in aquatic communities of rural and urban areas of West Bengal, India [8], with their prospective role in mosquito regulation being highlighted in few studies [4, 5]. While the adult species assemblages of Odonata have been demonstrated through several studies, the association of the larval stages and the macrophytes are yet to be fully described. Thus the present study was aimed at documentation of the odonate larval assemblages in relation to the hydrophytes using selected wetlands of West Bengal, India. The results are expected to bridge the gap between the taxonomic studies and ecological relevance of the odonate species in view of biological monitoring and conservation. Evaluation of insect species assemblages in wetlands are being highlighted for various reasons including the sustenance of ecosystem services. Owing to comparatively longer duration of the larval stages, odonate insects are considered as useful in environmental biomonitoring [9, 10]. The larval odonates can serve as bioindicator of habitat conditions owing to species specific preference for habitat conditions, both in lotic [11] and lentic ecosystems [12-16]. Therefore survey of Odonata communities can be considered as essential tool for characterizing and assessing the

habitat heterogeneity and hydrological features of the wetlands [17]. The results of the present study will justify the propositions pertaining to the utility of the odonate species assemblages in wetlands biomonitoring. Information for framing strategies for conservation of wetlands and odonate species in particular can be addressed on the basis of the odonate-hydrophytes relationship.

2. Materials and methods
2.1 Sampling sites
In the present study 93 different wetlands including ponds and temporary pools of varying size located in and around urban settlements of Kolkata, India and its peripheral districts Howrah, Hooghly, North 24 Parganas and South 24 Parganas were considered for sampling over a period of four months June to September 2013, coinciding with rainy season. The ponds and temporary pools harboured vegetations to different extent and were similar in the background landscape in the urban settings.

2.2 Sampling protocol
In order to collect dragonfly and damselfly larvae along with vegetations, random sampling was employed using an insect net (200 µm) placed in a circular iron frame with a diameter 32.5cm, attached with a long handle of 1.2 m [18]. In each pond, six different sites consisting of an area of 1m^2 was repeatedly sampled for vegetation and aquatic organisms. The total content from each site were placed in a plastic bag filled with water from the respective pond. Thus for each pond, collections of aquatic organisms and vegetations were placed in six plastic bags. The content of the plastic bags were emptied in an aquarium (37 X 30 X 30.5 cm^3) and subsequently to a plastic tub to aid in selection of the macroinvertebrates for identification. Although different groups of insects, snails, and oligochaetes were present in the collections, only the Odonata larvae were considered for counting and identification. For identification, the larvae were placed under a binocular microscope (Olympus, SZX7, Japan) and upon observation of the characters and comparison with the larval identification keys [19-22], the number of representative Odonata family and genus were recorded for each pond. For submersed and emergent vegetations, the plant parts that were found in the sampling area were collected, with or without root. Intact plants with root and shoot systems were considered as a single unit, for all types of vegetation. The identification of vegetations upto the species level was made following Cook [23]. The sampling protocol and the study design are mentioned in [19, 24-28] (Table 1).

For each pond, the pH, temperature, conductivity, total dissolved solids (TDS) and salinity were measured using a multiparameter tester (Multiparameter tester 35, Oakton instruments USA), and dissolved oxygen was measured using a Field DO kit (Oxygen test: Titrimetric method by Merck, Germany). The data on the water parameters were recorded from 30 cm distance from the bank at a depth of 20cm from the surface of the ponds.

Table 1: Outline of the study design and the sampling protocol followed in the assessment of the odonate-hydrophyte relationship in wetlands of West Bengal, India.

Parameters	Details	Remarks
Geographical range	South West Bengal, India, five districts	Howrah, Hooghly, Kolkata, N-24 Parganas and South- 24 arganas.
Habitat type and number	Wetlands ninety three	Range of area 400m^2 -560 m^2
Sampling	Random	6 / habitat = 93X6 samples/habitat
Duration	June to September 2010	Range=6 sampling/habitat-33 sampling/ habitat.
Vegetation identified	7 Species	Free floating, rooted emergent and benthic
Taxonomic levels of Odonata identified	Family and genus, following [19, 24]	Following the diagrams and the taxonomic keys shown in the book as well as the Australian museum
Water quality variables	Six	Temperature, dissolved oxygen, pH, conductivity, Total dissolved solids, Salinity.
Statistical analyses	Canonical correlation analyses	Program CANOCO Version [25-27]

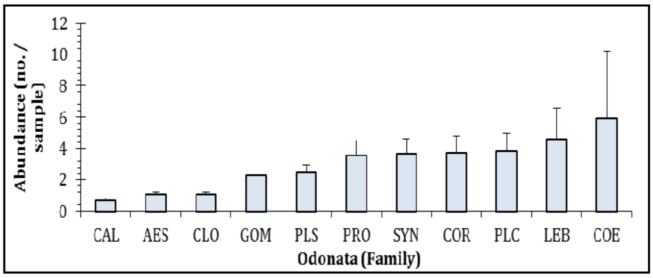
3. Data analysis
The data obtained on the Odonata, hydrophytes and water quality parameters were subjected to multivariate analyses to provide a classification [29], and ordination of Odonata against a gradient of hydrophytes [25-27]. The data on the hydrophytes was used as the explanatory variable to show the variations in the relative abundance of the Odonata larvae following the Canonical Correspondence Analysis (CCA) [25-27]. In CCA the hydrophytes were initially spread across the ordination axis and based on the affinity of the larvae to one or more of the hydrophytes, the secondary ordination is made justifying the association of the Odonata larvae against the hydrophytes available. Using the data of the relative abundance of the Odonata larvae at the family level and at the genus level, two CCA were carried out to comment on the ordination at family and genus levels respectively.

4. Results
In course of sampling of the wetlands, a total of 660 Odonate larvae under 10 families and 19 genera were obtained from the wetlands in different relative abundance (Table 2). Numerical representation of the families and genera of Odonata in each sample is shown in Fig 1 along with the hydrophytes observed in the wetlands. Among the hydrophytes, *Lemna minor* and *Wolffia* sp. dominated, while few specimens of *Sagittaria* sp. and *Ceratophyllum* sp. were observed but not in the proportion to be considered for analysis. Using the relative abundance of the Odonata larva the cluster analysis represented the similarity in appearance of the different families and genera as shown in Fig 2. The reflection of the similarity in the abundance provides an impression of the characteristic species assemblages to be encountered in the study area. However, owing to the sampling being done at low temporal variations, the relation among the different taxonomic groups of Odonata may show restricted variations. Interpretation of the temporal variations in the larval composition cannot be made from the present data.

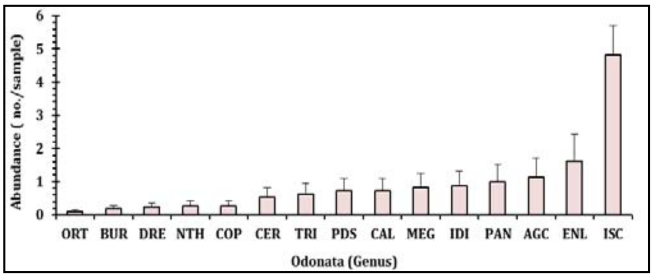
Application of the canonical correspondence analysis (CCA) using the data on the abundance of the Odonata larvae at the family level against the hydrophytes revealed that the four canonical axes explained 97% of variations in the species (Odonata family)and environment (hydrophytes) relation. The total inertia was 1.367 and the sum of all canonical eigenvalues was 0.141; showing significance at $P < 0.05$ level ($F = 1.519$ $P < 0.03$). The first canonical eigenvalue was ($\lambda_1 = 0.059$) was 1.54 times the second one ($\lambda_2 = 0.039$) and the first two canonical axes explained ~70% ($\lambda_1 = 43\%$ and $\lambda_2 = 27\%$) variance in the weighted averages of the species with respect to the hydrophytes (Fig 3). As represented through the arrows of the hydrophytes, it was apparent that the hydrophytes *Wolffia* (WOL) bears stronger correlation with the ordination axes while *Vallisneria* (VAL) bears weaker correlation with the ordination axes. It was apparent that the individuals of the families Platistictidae remained contrasting with Coenagrionidae, Synlestidae and Protoneuridae. Upon further resolution of the data upto the genus level, the CCA analysis revealed further distinction, in that the four canonical axes explained 93% of variations in the species (Odonata genus) and environment (hydrophytes) relation. The total inertia was 2.33 and the sum of all canonical eigenvalues was 0.229, showing significance at $P < 0.05$ level ($F = 1.398$; $P < 0.03$). The first canonical eigenvalue was ($\lambda_1 = 0.085$) was 1.46 times the second one ($\lambda_2 = 0.058$) and the first two canonical axes explained ~63% ($\lambda_1 = 37\%$ and $\lambda_2 = 26\%$) variance in the weighted averages of the species with respect to the hydrophytes (Fig 4). In this analysis the hydrophytes *Marsilia* (MAR) exhibited a stronger correlation with the ordination axes, while *Lemna* (LEM) showed weaker correlation with the ordination axes. The results remained different from the family level analysis possibly because of the contrasting preferences by the species of dragonfly (Anisoptera) with that of the damselfly (Zygoptera). Larva of the Odonata genus *Neurothemis* exhibited contrasting ordination with *Drepanosticta* as well as other genera. However, among the damselflies *Enalagma* and *Ischnura* remained similar to one another but contrasting to the other common genus *Ceriagrion* (CER) (Fig 4). Although ordination of the family and the genus levels of Odonata could be achieved at a satisfactory level, taxonomic resolution at the species level could have provided a better ordination. We assume both the low taxonomic resolution and the restriction of the samples from the urban and peri-urban localities to be the reasons for lower degree of ordination of the larval Odonata against the hydrophytes in the wetlands sampled.

Table 2: The proportional representation of the Odonata family and genus observed in the wetlands in West Bengal, India. (N= 662 specimens in 93 wetlands)

Odonata -Family	n_i	Odonata -Genus	n_i
Calopterygidae (CAL)	0.002	Aeshna(AES)	0.002
Aeshnidae(AES)	0.003	Anax(ANX)	0.002
Chlorocyphidae (CLO)	0.003	*Neurobasis* (NEU)	0.002
Gomphidae (GOM)	0.014	*Rhinocypha* (RHI)	0.003
Platystomatidae (PLS)	0.017	*Orthetrum*(ORT)	0.008
Protoneuridae (PRO)	0.051	*Burmagomphus* (BUR)	0.014
Synlestidae (SYN)	0.059	*Drepanosticta* (DRE)	0.017
Corduliidae (COR)	0.062	*Neurothemis* (NTH)	0.020
Platycnemidae (PLC)	0.071	*Copera* (COP)	0.020
Libellulidae (LEB)	0.145	*Ceriagrion* (CER)	0.038
Coenagrionidae (COE)	0.574	*Trithemis* (TRI)	0.045
		Prodasineura (PDS)	0.051
		Calicnemia (CAL)	0.051
		Megalestes (MEG)	0.059
		Idionyx (IDI)	0.062
		Pantala (PAN)	0.071
		Agriocnemis (AGC)	0.082
		Enallagma (ENL)	0.115
		Ischnura (ISC)	0.341



(a)



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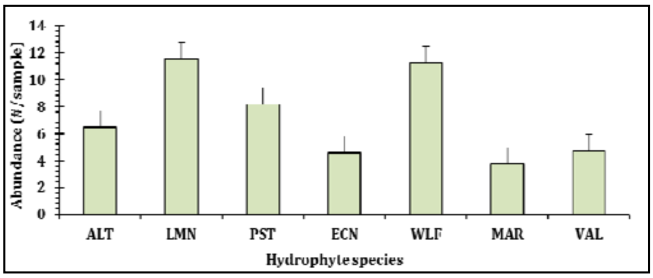
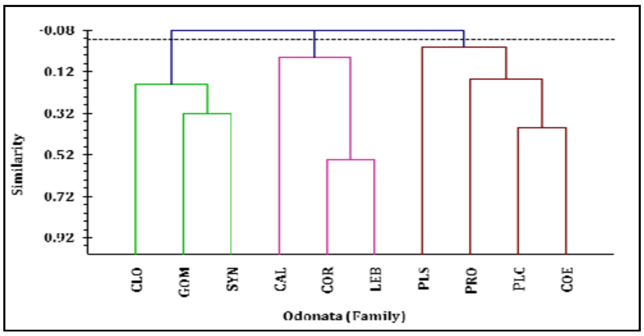


(c)

Fig1: The relative abundance of the Odonate larvae in each sample – (a) family wise, and (b) genus wise and the (c) macrophytes observed in the wetlands of West Bengal, India.



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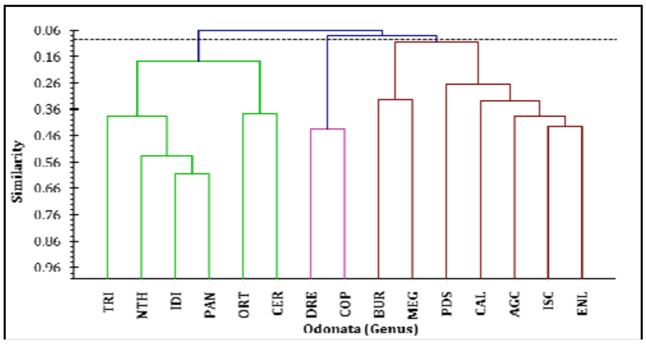

(b)

Fig 2: The cluster analysis to represent the relative similarity of the different families (a) and genera (b) depending on the relative abundance in each sample collected from the wetlands of West Bengal.

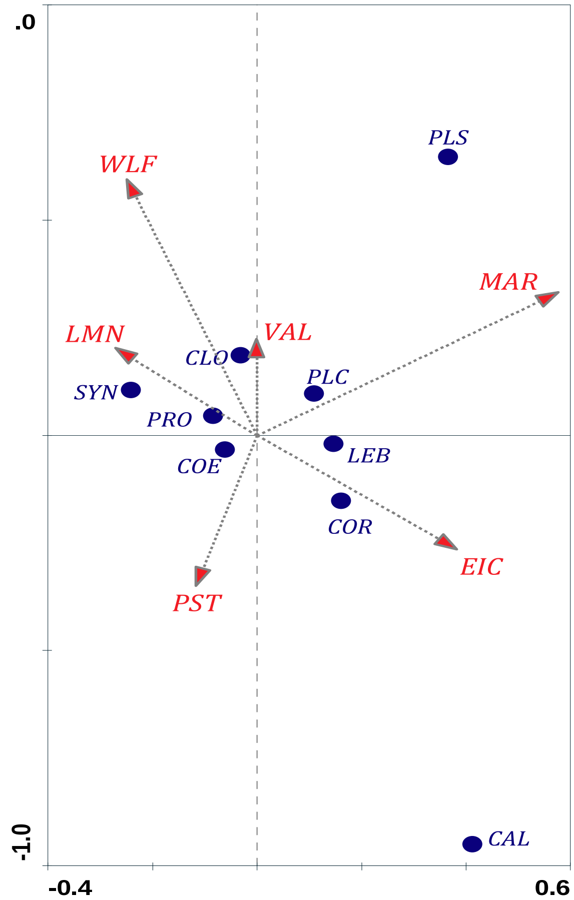

Fig 3: The CCA biplot representing the ordination of the Odonata (Family) against the different hydrophytes collected from 93 different wetlands of West Bengal, India.

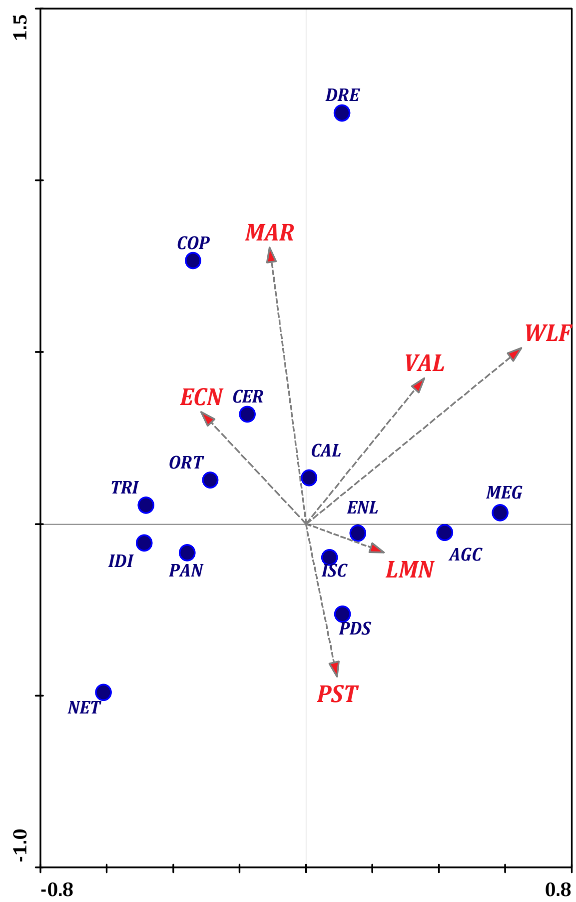

Fig 4: The CCA biplot representing the ordination of the Odonata (Genus) against the different hydrophytes collected from 93 different wetlands of West Bengal, India.

5. Discussion

The sampling study of the selected wetlands of the southern districts of West Bengal revealed a strong correspondence between macrophytes and the Odonata larva. Although restricted to the genus level, larva of at least 19 different genera was encountered in course of the study. The representative genera like *Ceriagrion*, *Enallagma*, *Ischnura* (damselfly) and the *Aeshna* (dragonfly) remained common in the wetlands where the vegetations were dominated by *Wolffia*, *Marsilea*, *Pistia*, *Eichhornia* and Vallisneria. Variations in the relative abundance of the different genera and families of Odonata in the present study are comparable to the studies made elsewhere in India [3, 6, 7] and other countries. However, in Indian context the studies were restricted to the collection of the adult specimens which is expected to differ in terms of the species composition. The observed genera of Odonata complied with the earlier observations of West Bengal, though the lack of taxonomic resolution to the species level restricts comparison with the observations made in other regions of India. While majority of the studies on Odonata is restricted to the estimation of the adult species assemblages, the present information is a pioneer effort to document the larval assemblages in the context of the vegetation available in the freshwater wetlands. The larval assemblages of Odonata is influenced by the hydrophytes of the water bodies as evident from the studies on the lakes in USA [7] and rivers in African savanna [11]. In both the instances the variations in the relative abundance of the larval Odonata could be explained considerably through the available hydrophytes. The density of the hydrophytes in each site influenced the availability of the Odonata larvae to a significant extent in contrast to the open sites without the hydrophytes [7]. Although not assessed in the present study, the results comply with the influence of the hydrophytes on the larval Odonata availability in the wetlands considered in the present study. Hydrophytes like *Stratiotes aloides* provide protection to the Odonata larva against the fish predation, while, in general the predatory efficiency of the larva may be reduced as observed for the predatory potential against the mosquito and chironomid larvae. Increased complexity of the habitats while protecting the odonate larvae may also render them less efficient as predators. Nonetheless, the hydrophytes bear considerable influence on the distribution of the larval Odonata in the microhabitats of the wetlands.

Alike other aquatic insects, the habitat requirements of Odonata are highly species-dependent [30]. Efforts to highlight the role of the hydrophytes in the distribution and species assemblages of Odonata in lentic system [31] and lotic systems have been made to facilitate strategies for conservation. Since alteration to the habitat conditions without the hydrophytes reduce the Odonata diversity [32, 11], the preservation of the wetland hydrophytes remain an integral part to enhance the sustenance of the Odonata in any geographical areas. Considering the ecological role of the larval Odonata in the regulation of the diversity of the aquatic macroinvertebrates and regulation of the medically important species like, mosquitoes and snails [3, 5] conservation planning for sustaining population of different Odonata species is essential. Apart from the aquatic breeding sites, the conservation of the Odonata requires appropriate terrestrial landscapes for the survival of the adults [33, 34]. The role of the hydrophytes is important in determining the foraging sites of the adults besides providing suitable habitat conditions for the growth of the larva. As documented in the present study the role of the hydrophytes in determination of the assemblage pattern of the

Odonata should be prioritized to sustain the ecological functions and enhance the quality of the aquatic insect assemblages. Aquatic insect communities of wetlands are diverse in nature with plenty of representatives from larval and adult Hemiptera, Coleoptera and Neuroptera that feed on Ephemeroptera and Diptera apart from other prey species. In order to enhance the stability of the Odonata populations in the wetlands the interactions among the different groups of aquatic insects should be carried out to identify the species combinations suitable for the wetlands. In the present study a preliminary observation is being depicted using the hydrophytes as the determining factor for the Odonata species distribution in selected wetlands. Further studies should be undertaken to understand the interaction between the different Odonata groups and with other predatory aquatic insects for enhancing the strategies for conservation of Odonata in the geographical region concerned.

6. Acknowledgements

The authors are thankful to the respective Heads, Department of Zoology, University of Calcutta, Kolkata, and The University of Burdwan, Burdwan, for the facilities provided including DST-FIST, Government of India.

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