

Dragonfly in Bogani Nani Wartabone National Park North Sulawesi

MEIS J. NANGOY

ORCID No. 0000-0003-0064-3623

mnangoy@unsrat.ac.id

Animal Science, Faculty, Sam Ratulangi University,
Kampus Bahu Street, Manado 95115

RONI KONERI

ORCID No. 0000-0001-5972-0799

ronicaniago@unsrat.ac.id

Department of Biology, Faculty of Mathematics and Natural Sciences,
Sam Ratulangi University,
Kampus Bahu Street, Manado 95115

ABSTRACT

As a group of freshwater invertebrates, dragonflies (Odonata) are commonly used as ecological indicators of freshwater ecosystems. This study analyzes the diversity of Odonata in various types of habitat at Bogani Nani Wartabone National Park, North Sulawesi, Indonesia. Sampling was conducted over three months in three habitat types, viz (primary forest, secondary forest, and agricultural land). Samples were taken along the transect line using a sweep net. Samples were collected from 9:00 am to 03:00 pm during the periods that odonates are most active. Identification was based on external morphological characteristics using the relevant guide. In total, 1235 specimens of Odonata were obtained belonging to 19 species, 17 genera, and 7 families. Abundance, and richness of dragonfly species positively correlated with air temperature, but negatively correlated with humidity and vegetation cover. Based on the results

of this study, three species of dragonflies, viz *Nososticta flavipennis*, *Rhinocypha frontalis*, and *Teinobasis* sp are found only in primary forests and are not found in other habitats. They can be used as indicators of forest health. The highest abundance, richness, diversity, and evenness of dragonfly species are found on agricultural land, while the lowest were found in secondary forests.

Keywords: Abundance, richness, evenness, *Rhinocypha frontalis*, primary forest

INTRODUCTION

Odonata is a group of insects with global diversity about 6000 species and subspecies, 630 genera and 28 families (Sharma et al. 2007). Dragonflies are grouped into two sub orders, Zygoptera (2739 species and 19 families) and Anisoptera (2941 species and 12 families) as well as about 1000 to 1500 undescribed species (Van Tol 1992). Diversity at species and family level is highest in the tropics. Twelve of the 31 families are confined to tropical forests. In Sulawesi there are at least 124 species, 55 of which are island endemics. Based on these data, Sulawesi endemismity ranks fifth in the world and Indonesia is second (Van Tol 1987).

Various studies have investigated the relationship between odonata diversity and environmental factors and land use such that northern Peninsular Malaysia (Siregar et al. 2005); distribution and diversity of dragonflies in Kerian River Kedah, Malaysia (Ameilia et al. 2006); in the Sekayu Forest, Terengganu, Malaysia (Afzan et al. 2006); Andin Similipal Tiger, India (Sunit et al. 2012). Odonata biodiversity of the Argentine Chaco biome (Von ellendieder 2010).

Bogani Nani Wartabone National Park a conservation area in Indonesia that created in 1991, and has an area of 287.115 hectares. It has three main functions: protection of life support systems, preservation of plants and wildlife as well as sustainable use of natural resources and ecosystems. Presently, around the Park already experience conversion of forest into agricultural land. Deforestation of tropical rain forest will also have a particularly strong impact on biodiversity. Frequent anthropogenic disturbances here lead to immediate reduction in diversity and extinction of many arthropods (Dolny et al. 2012). These disturbances are imminently the most threatening to Odonata diversity and will potentially result in extinction of numerous species (Orr 2004; Clausnitzer et al. 2009).

There is very limited knowledge on the diversity of dragonflies as a biological control and bio-indicators of the environment in Bogani Nani Wartabone National Park, although these insects play a crucial role in the ecosystem.

OBJECTIVES OF THE STUDY

This study aimed to determine dragonfly species that can be used as biological control agents and bio-indicators of the environment in Bogani Nani Wartabone National Park, North Sulawesi.

MATERIALS AND METHODS

Study Site

This study was conducted from May-July 2014 in Bogani Nani Wartabone National Park, North Sulawesi. The research involved sampling dragonflies and measuring of physical-chemical factors. Sampling was executed in three habitat types, viz., primary forest, secondary forest and agricultural land. In each habitat, three transect lines with a length of 1000 m were established. Transect lines were created around streams to include these three habitat types (Table 1).

Collection and Identification

Samples were taken along the transect line using a sweep net. Samples were collected from 9:00 am to 03:00 pm during the periods that odonates are most active. Dragonflies were captured, put into envelopes, placed into bottles containing acetone for 12 hours then dried in the sun finally then stored in triangular paper envelopes with wings folded into position along the body (Kalkman and Orr 2013). Identification was based on external morphological characteristics using the relevant guide (Borror et al. 1996; Watson and Farrel 1991; Miller 1995; Liefertinck 1949). Photos were taken and sent to Naturalis Biodiversity Center Leiden for confirmation of identifications. Measurement of environmental factors include water temperature, air temperature, relative humidity (Rh) and acidity (pH). Measurements of environmental factors were done with the assumption that differences in the type of habitat conditions would cause different microclimate conditions affecting the population abundance of dragonflies.

Data Analysis

The data analyzed include the abundance of species (n), species richness (S), species diversity (H) and species evenness value (E). Determining the level of species diversity was done using the diversity index (H) by Shannon & Wiener

(Magurran 1988), with the following formula:

$$(H') = - \sum (Pi) (\ln Pi)$$

Specification:

Pi = proportion of each species;

ln = natural logarithm (natural number)

To determine the level of species evenness we used the Shannon evenness index (E), as follows:

$$E = H / \ln (S);$$

S = number of species (Magurran 2004).

Data processing used the program STATISTICA Version 6, a one-way ANOVA (one-way ANOVA). Tukey's test at the 95 % confidence level was used to determine differences in species richness, abundance of species, species diversity and evenness values of species in each habitat type (Statsoft 2001; Koneri 2016). To determine the relationship between species diversity with the value of physical - chemical parameters of environment we used a Spearman correlation test.

RESULTS AND DISCUSSION

Dragonflies found in the Bogani Nani Wartabone National Park consisted of the sub-orders Anisoptera (Figure 1) and Zygoptera (Figure 2), seven families, 17 genera, 19 species and 1235 individuals. Anisoptera were represented with two families (Gomphidae and Libellulidae), 9 genera, 10 species and 408 individuals and the suborder Zygoptera with five families (Calopterygidae, Chlorocypridae, Coenagrionidae, Lestidae and Protoneuridae), nine species, eight genera and 827 individuals (Table 2).

The highest relative abundance was shown by the Coenagrionidae, followed by Libellulidae. Families with the lowest abundance are Gomphidae (0.08%) and Protoneuridae (0.16%) (Figure 3). Libellulidae were most species with 9 species, then Coenagrionidae and Chlorocypridae (with three species each). Gomphidae, Lestidae and Protoneuridae were represented with only one species.

Dragonflies species with the most individuals are *Pseudagrion* sp, and *Celebothemis delectollei*, *Ictinogomphus celebensis* (one individual) and *Nannophya pygmaea* (2 individuals) were relatively scarce (Table 2). Agricultural land had the highest number of specimens, and then primary forest (Table 2).

The average values of abundance, richness, diversity and evenness were highest for agricultural land, followed by primary forest while the lowest value was

observed in secondary forest. Statistical test results showed an average abundance of dragonflies significantly differed between habitats ($P < 0.05$) (Figure 4). Odonata species richness and the diversity index (H') were highest on agricultural land habitat, followed by primary forest habitat, and the lowest is secondary forest ($S = 13.33$ and $H' = 2.18$). Statistical test results showed that richness and diversity of dragonfly species were significantly different between habitats ($P < 0.05$) (Figure 2). The highest dragonfly species evenness is on agricultural land and the lowest in secondary forest habitat. Statistical test results showed dragonfly species evenness did not differ significantly between habitats ($P > 0.05$) (Figure 4).

The results of the Spearman correlation test between community structures of dragonflies with physical parameters are presented in Table 3. Dragonfly species abundance positively correlated with air temperature. The higher the ambient temperature, the higher abundance of dragonfly species found. Dragonfly species richness was negatively correlated with humidity, and vegetation cover. The lower the vegetation cover, the higher the number of dragonfly species found. Dragonfly species diversity is negatively correlated with air temperature, humidity, and vegetation cover. Based on the correlation value we found that the greatest species diversity correlated with vegetation. The lower the vegetation cover, the higher the diversity of dragonfly species found.

Dragonfly species evenness correlated negatively with air temperature and humidity. Based on the correlations found, most large species evenness correlated with air temperature. The higher the temperatures the more evenness of species found.

In among the three studied habitats, there are three species of Zygoptera species found only in primary forest. These species can be used as bio-indicators of forest health are *Nososticta flavipennis*, *Rhinocypha frontalis*, and *Teinobasis* sp.

Libellulidae and Coenagrionidae abundance were the most abundant members of suborder Anisoptera and Zygoptera, respectively. Libellulidae is the largest family in the suborder Anisoptera; most species are widespread and show adaptability (Patra et al. 2016). Also Shende and Patil (2013) found that family Libellulidae is most commonly found. The Coenagrionidae is the largest family in the suborder Zygoptera and spread evenly across the world (Orr 2003). Species Coenagrionidae most commonly found in stagnant water (Kalkman and Orr 2013). The high adaptability of species of the family Libellulidae and Coenagrionidae results in dominance of this family in many habitat types. The abundance of Libellulidae (Anisoptera) and Coenagrionidae (Zygoptera) in the present study might be due to their short life cycle and tolerant to wide range of

habitats (Arulprakash and Gunathilagarai 2010).

Dragonfly abundance is high on agricultural land due to the high light intensity and a lack of vegetation cover around (Anaya et al. 2011). Dragonflies have a habit of basking in the sun to warm the body and to strengthen the muscles of their wings to fly (Susanti 1998).

High species richness and diversity (H') on agricultural land are affected by the vegetation along streams, the availability of food, and light intensity. Agricultural land has high light intensity because it is more open and existing vegetation around the streams are in the form of grass, and the use of land for agriculture such as maize (*Zea mays*), rice (*Oryza sativa*), and coconut (*Cocos nucifera*) also has attracted high numbers of pest insect that are eaten by dragonflies (Susanti 1998).

The low evenness dragonfly species in secondary forests show that there are several species of dragonflies dominate in the population. Some species of dragonflies that dominate are *Pseudagrion* sp and *Celebothemis delectolei*. The dominance of these species is related to high environmental adaptability (Orr 2003).

The positive correlation between the abundance of species and the air temperature is due to the dragonfly's habit of sunbathing to warm the body and strengthen the muscles of the wing (Susanti 1998; Baruah and Saikia 2015). Dragonfly species abundance was negatively correlated with humidity and vegetation cover. The lower the humidity and vegetation cover, the higher abundance of dragonfly species found. Adult dragonflies must keep their body temperature independent of environmental temperature to maintain activities vigor for flying. To maintain its body temperature dragonflies must thermoregulate. Furthermore, to initiate flight is necessary increase the temperature of flight muscles (Fullan et al. 2008).

Dolny et al. (2011) reported that there are many Zygoptera missing from forests that have been disturbed or that have experienced reforestation. The presence of such species can indicate the healthy condition of the forest. Primary forests are more commonly inhabited by characteristic species, because the environmental capacity of primary forests such as temperature, moisture, and vegetation cover further supports the existence of such species. Six species of primary forests were not found in secondary forest habitats and agricultural. Population sizes of the damselfly species significantly decrease with habitat degradation (Dolny et al. 2011).

The Dragonfly species that are only on agricultural land but not found in

other habitats are two species. They consist under sub-order Anisoptera namely *Neurothemis ramburii* and *Neurothemis stigmatizans* and the species is found only in secondary forests and other habitats *I. celebensis* (Gomphidae, Anisoptera).

CONCLUSION

The highest abundance, richness, diversity and evenness of dragonfly species are found on agricultural land, while the lowest were found in secondary forests. Abundance and richness of dragonfly species were positively correlated with air temperature, but negatively correlated with humidity and vegetation cover. Based on the results of this study, three species of dragonflies, viz *Nososticta flavipennis*, *Rhinocypha frontalis*, and *Teinobasis* sp. are found only in primary forests and are not found in other habitats. They can be used as indicators of forest health.

ACKNOWLEDGEMENTS

The authors would like to thank the Directorate General of Higher Education, for funding this research through grants Fundamental 2014. Our thanks also go to the Director of Nani Wartabone National Park North Sulawesi and its staff for permission and the facilities provided while conducting the research and Dr. Jan van Tol (Naturalis Biodiversity Center Leiden) for assistance with species identification.

LITERATURE CITED

- Arulprakash R, Gunathilagaraj K. 2010. Abundance and diversity of odonata in temporary water bodies of Coimbatore and Salem districts in Tamil Nadu. *Journal of Threatened Taxa.*, 2(8): 1099-1102.
- Afzan AW, Julia J, Amirrudin A. 2006. Diversity and distribution of dragonflies (Insecta: Odonata) in Sekayu Recreational Forest, Terengganu. *J. of Sust. Scien. and Manag.*, 1 (2): 97-106.
- Ameilia ZS, Che Salmah MR, Hassan AA. 2006. Diversity and distribution of dragonfly (odonata: insecta) in the Kerian River Basin, Kedah-Perak, Malaysia. *USU Repository*: 14 p.

- Anaya JAG, Gutierrez RN, Campbell WB. 2011. Diversity and distribution of odonata (insecta) larvae along an altitudinal gradient in Coalcomán mountains, Michoacán, Mexico. *Rev. Biol. Trop.*, 59 (4): 1559-1577.
- Baruah C, Saika PK. 2015. Abundance and diversity of odonates in different habitats of Barpeta District, Assam, India. *Int. Res. J. Biological Sci.*, 4(9): 17-27.
- Borror BJ, Triplehorn CA, Johnson NF. 1996. An introduction to the study of insects.ed. Ke-6. Gajah Mada University Press, Yogyakarta. Indonesia.
- Clausnitzer V, Kalkman VJ, Ram M, Collen B, Baillie JEM, Bedjanič M, Darwall WRT, Dijkstra KDB, Dow R, Hawking J, Karube H, Malikova E, Paulson D, Schutte K, Suhling F, Villanueva R, von Ellenrieder N, Wilson K. 2009. Odonata enter the biodiversity crisis debate: the first global assessment of an insect group. *Bio. Conserv.*, 142: 1864-1869.
- Dolny A, Bárta D, Lhota S, Rusdianto, Drozd P. 2011. Dragonflies (odonata) in the bornean rain forest as indicators of changes in biodiversity resulting from forest modification and destruction. *Tropical Zoo.*, 24: 63-86.
- Dolny A, Harabis F, Bárta D, Lhota S, Droz P. 2012. Aquatic insects indicate terrestrial habitat degradation: changes in taxonomical structure and functional diversity of dragonflies in tropical rainforest of East Kalimantan. *Tropical Zoo.*, 25 (3):141–157.
- Fulan JA, Raimundo R, Figueiredo D. 2008. Habitat characteristics and dragonflies (odonata) diversity and abundance in the Guadiana River, Eastern of the Alentejo, Portugal. *Boln. Assoc. Esp. Ent.*, 32 (3-4): 327-340.
- Kaize J, Kalkman V. 2011. Records of dragonflies (odonata) from Kabupaten Asmat and Kabupaten Mappi (Papua, Indonesia). *Suara Serangga Papua*, 5 (3): 99-107.
- Kalkman V, Orr AG. 2013. Field guide to the damselflies of New Guinea. *Brachytron*, 16: 3-120.

- Koneri R, Maabuat PV. 2016. Diversity of butterflies (Lepidoptera) in Manembo-Nembo Wildlife Reserve, North Sulawesi, Indonesia. *Pak. J. Biol. Sci.*, 19: 202-210.
- Lieftinck MA. 1949. The dragonflies (odonata) of New Guinea and Neighbouring Islands. Part VII. Results of Third Archbold Expedition 1938-1939 and the Le Roux Expedition 1939 to Netherlands New Guinea (II. Zygoptera). *Nova Guinea New Series*, 5: 1-271.
- Magurran AE. 1988. *Ecological diversity and its measurements*. London: Croom Helm Limited. London.
- Magguran AE. 2004. *Measuring biological diversity*. Malden: Blackwell Publishing.
- Miller PL. 1995. *Dragonflies, the Queen's College*, Oxford.
- Orr AG. 2003. *A guide to the dragonflies of borneo their identification and biology*. Natural History Publications (Borneo). Kinabalu.
- Orr AG. 2004. Critical species of odonata in Malaysia, Indonesia, Singapore and Brunei. *Int. J. of Odon.*, 7(2): 371-384.
- Patra D, Roy S, Chowdhury S. 2016. Diversity and abundance of odonata fauna in Midnapore and surrounding areas, West Midnapore, West Bengal. *J. of Ento. and Zoo. Stud.*, 4(6): 553-558.
- Sharma G, Sundararaj R, Karibasvaraja LR. 2007. Species diversity of odonata in the selected provenances of sandal in Southern India. *Zoos' Print J.*, 22 (7): 2765-2767.
- Shende VA, Patil KG. 2013. Diversity of dragonflies (Anisoptera) in Gorewada International Bio-Park, Nagpur, Central India. *Arthropods*, 2(4): 200-207.
- Siregar AZ, Rawi CS, Ahmad AH. 2005. The diversity of odonata in relation to ecosystem and land use in Northern Peninsular Malaysia. *J. Ilmiah Pertanian Kultura*, 40 (2): 106-116.

StatSoft. 2001. Statistica for windows, 6.0 statsoft Inc. Tulsa: Oklohoma.

Sunit Kr D, Ahmed RA, Sajan SK, Dash N, Sahoo P, Mohanta P, Suhu HK, Rout SD, Dutta SK. 2012. Diversity, distribution and species composition of odonates in buffer areas of Similipal Tiger Reserve, Eastern Ghat, India. *Acad.J. of Ent.*,5 (1): 54-61.

Susanti S. 1998. The field guide dragonflies. Puslitbang Biologi-LIPI. Bogor.

Van Tol J. 1987. The odonata of Sulawesi and adjacent islands. Part 2. The Genus Brauer on Sulawesi. *Zoologische Mededelingen*, 61: 160-176.

Van Tol J. 1992. An annotated index to names of odonata used in publications by M. A Liefstinck, *Zoologische Verhan Deligen*, National Natuur historisch Museum Leiden, The Netherlands.

Von Ellenrieder N. 2010. Odonata biodiversity of the Argentine Chaco biome. *Int. J. of Odon.*, 13 (1): 1-25. DOI:10.1080/13887890.2010.9748357.

Watson JAL, Farrel AFO. 1991. Odonata. In (naoman et.al.eds.) *insect of Australia* Vol.1. MelboumeUniversity Press.

APPENDICES

Table 1. Habitat characterization

Parameters	Primary Forest	Secondary Forest	Agriculture Land
GPS coordinates	Transect 1 00°34' 33.87"N, 123°53'58.02"E.	Transect 1 1: 00°33'44.99"N, 123°54'10.77"E.	Transect 1 00°33'33.32"N, 123°54'08.33"E.
	Transect 2 00°34'37.33"N, 123°54'00.64"E.	Transect 2 (00°33'50.49"N, 123°54'10.55"E.	Transect 2 00°33'32.23"N, 123°54'03.38"E.
Altitude	Transect3: 00°34'43.02"N, 123°53'59.73"E.	Transect 3: 00°33'39.57"N, 123°54'10.28"E.	Transect 3 00°33'37.44"N, 123°53'53.30"E.
	Temperature 241 m-289 m	210 m - 225 m.	202 m-223 m
Humidity	26°C-30°C	26°C-33°C	28°C-32°C
vegetation cover	65%-74%.	65%-81%.	42%-70%.
Vegetation	60-85%	40-55%.	20-30%.
	<i>Myrsine fragrans</i> , <i>Garcinia mangostana</i> , <i>Diospyros minahasae</i> , <i>Pometia tomentosa</i> , <i>Micheila champaca</i> , <i>Diospyros pilosanthera</i> , <i>Bambusa</i> sp, <i>Ficus</i> sp, <i>Calamus</i> sp, <i>Pandanus</i> sp.	<i>Bambusa</i> sp, <i>Ficus</i> sp, <i>Pennisetum purpureum</i> , <i>Piper aduncum</i> , <i>Macaranga</i> sp, <i>Calamus</i> <i>nollis</i> , <i>Pterospermum</i> <i>celebicum</i> , <i>Polyalthia</i> <i>glauca</i> , <i>Diospyros</i> <i>minahasae</i> .	<i>Bambusa</i> sp, <i>Cynodon</i> <i>dactylon</i> , <i>Saccharum</i> <i>spontaneum</i> , <i>Amaranthus</i> <i>spinosus</i> , <i>Piper aduncum</i> , <i>Cocos nucifera</i> , <i>Oryza</i> <i>sativa</i> , <i>Zea mays</i> , <i>Capsicum</i> sp, <i>Solanum</i> sp. <i>Artocarpus integra</i> , <i>Musa</i> <i>paradisica</i> , <i>Pearcea</i> <i>americana</i> , <i>Theobroma</i> <i>cacao</i> .

Table 2. Number of sub orders, families and species of dragonflies found in three types of habitat in Nani Wartabone National Park.

No	Sub Order/Family/Species	Primary forest		Secondary forest		Agriculture land		Total	
		Σ	%	Σ	%	Σ	%	Σ	%
Anisoptera									
I Gemphidae									
1	<i>Ictinogomphus celebensis</i>	0.00	0.00	1.00	0.08	0.00	0.00	1.00	0.08
II Libellulidae									
2	<i>Actonema panorpetae</i>	0	0	0	0	0	0.73	0	0.73
3	<i>Celebothebis delectabilis</i>	46	3.72	40	3.24	67	5.43	153	12.39
4	<i>Diplacina</i> sp	24	1.94	6	0.49	46	3.72	76	6.15
5	<i>Neurothebis pygmaea</i>	0	0	2	0.16	17	1.38	19	1.54
6	<i>Neurothebis nambarti</i>	0	0	0	0	29	2.35	29	2.35
7	<i>Neurothebis strigatissans</i>	0	0	0	0	23	1.86	23	1.86
8	<i>Orethebis prasinosa</i>	1	0.08	33	2.67	37	3	71	5.75
9	<i>Pantala flavescens</i>	0	0	0	0	13	1.05	13	1.05
10	<i>Trithemis formosa</i>	0	0	0	0	14	1.13	14	1.13
Zygoptera									
III Calopterygidae									
11	<i>Neurobasis kampi</i>	88.00	7.13	0.00	0.00	8.00	0.65	96.00	7.77
IV Chlorocyptidae									
12	<i>Libellago xanthocypana</i>	6	0.49	31	2.51	50	4.05	87	7.04
13	<i>Libellago daviesi</i>	19	1.54	5	0.4	25	2.02	49	3.97
14	<i>Rhinocypha frontalis</i>	94	7.61	0	0	0	0	94	7.61
V Coenagrionidae									
15	<i>Agrionessentia femina</i>	0	0	12	0.97	59	4.78	71	5.75
16	<i>Pseudagrion</i> sp	25	2.02	186	15.06	158	12.79	369	29.88
17	<i>Tenobasis</i> sp	36	2.91	0	0	0	0	36	2.91
VI Lestidae									
18	<i>Lestes</i> sp	0.00	0.00	5.00	0.40	18.00	1.46	23.00	1.86
VII Protoneturidae									
19	<i>Nocosticta flavipennis</i>	2.00	0.16	0.00	0.00	0.00	0.00	2.00	0.16
Grand Total		341.00	27.61	321.00	25.99	573.00	46.46	1235.00	100.00

Table 3. Correlations between Community Structure Dragonflies with Chemical – Physical Parameters environment

Community structure	Temperature	Humidity	Vegetation cover
Abundance	0.36	-0.60	-0.63
Richness	0.19	-0.58	-0.74
Diversity	-0.22	-0.39	-0.27
Evenness	-0.35	-0.20	0.09

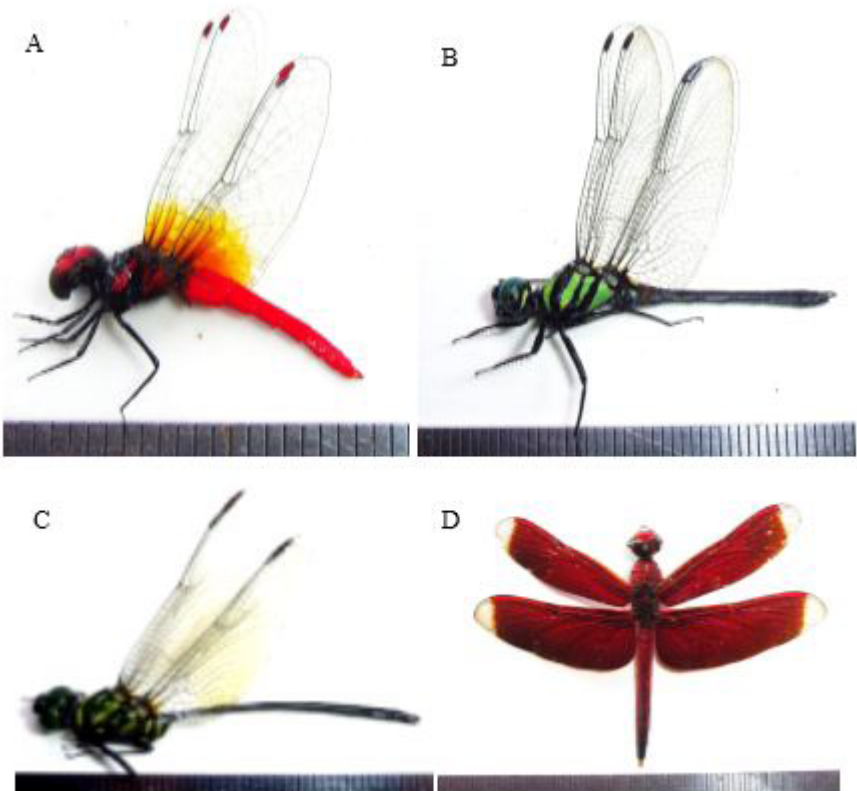


Figure 1. Photographs of Anisoptera (A-D). A. *Nannophya pygmae*, B. *Diplacina* sp, C. *Celebothermis delectollei*, D. *Neurothermis stigmatizans*

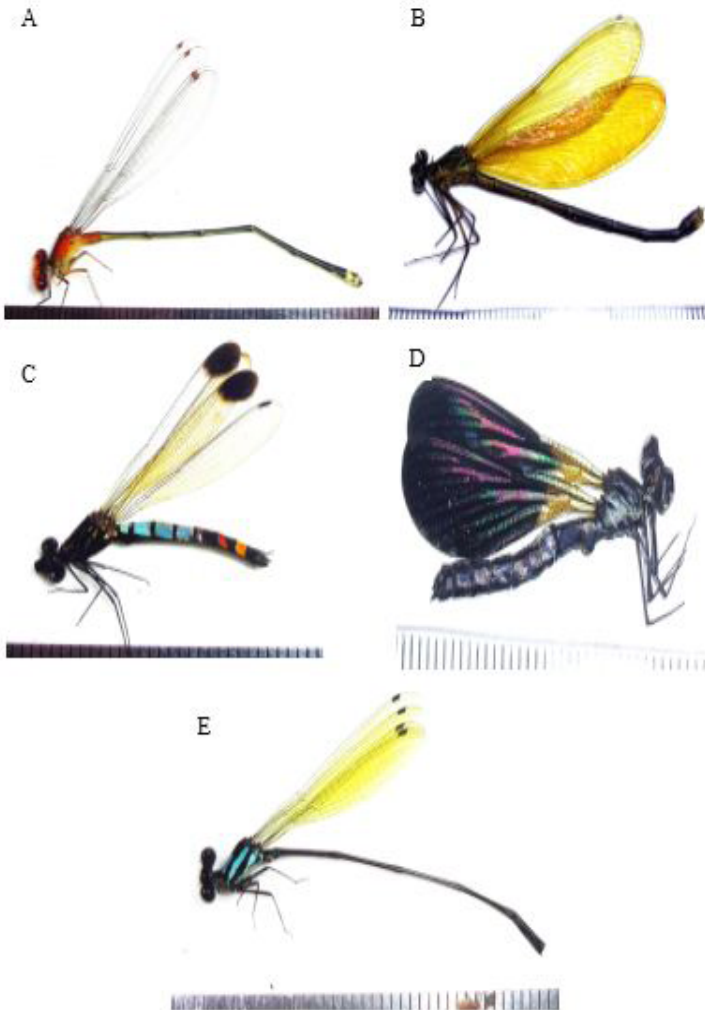


Figure 2. Photographs of Zygoptera (A-E). A. *Pseudagrion* sp, B. *Neurobasis kaupi*, C. *Libellago xanthocyanea*, D. *Rhinocypha frontalis*, E. *Nososticta flavipennis*

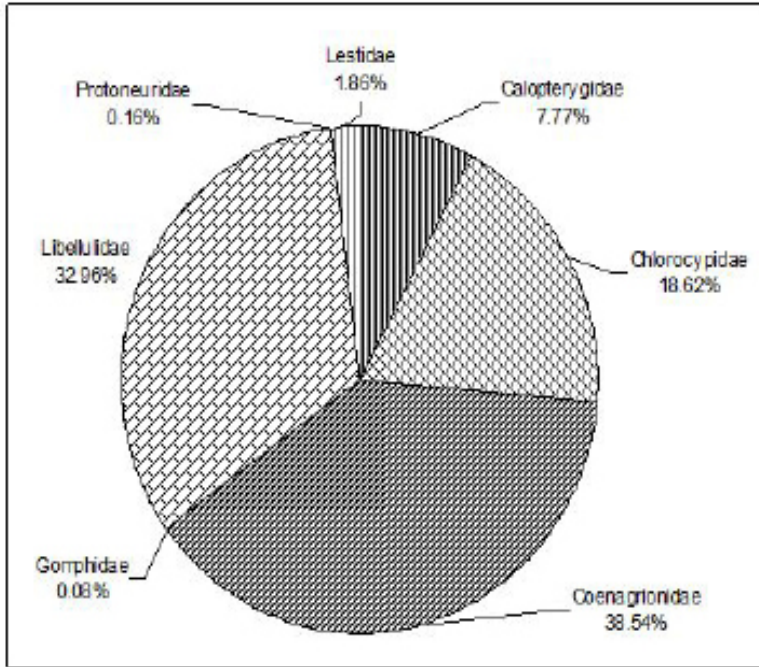


Figure 3. Number of dragonfly families found in three types of habitat in Nani Wartabone National Park.

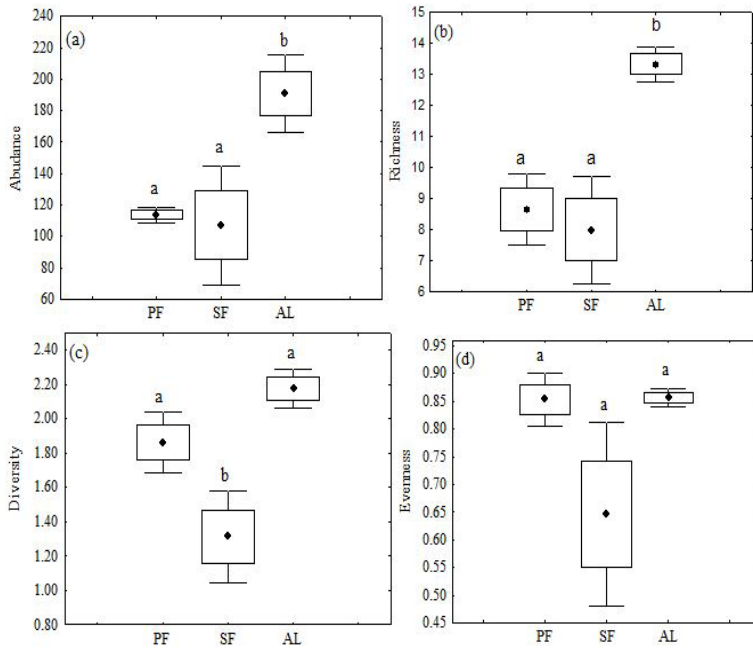


Figure 4. Abundance (a), Richness (b), diversity (c) and species evenness (d) dragonfly on three types of habitat (PF: primary forest; SF: secondary forest; AL: Agriculture land), (●) : on average , (□) : \pm standard error (\pm SE) , (▭) : \pm standard deviation (\pm SD), the same letter on the same figure not significantly different according to Tukey's test at the 95% confidence level)