Tropical Ecology **58**(1): 177-191, 2017 ISSN 0564-3295

www.tropecol.com

Dynamics of aquatic organisms in a rice field ecosystem: effects of seasons and cultivation phases on abundance and predator-prey interactions

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Abstract: The influence of different rice growing seasons and rice cultivation phases on aquatic organisms was investigated in the Manik Rambung rice field (MRRF) ecosystem in North Sumatera. Composite collections of core samples and aquatic net samples from four rice growing seasons and five cultivation phases (fallow, plough, transplanting-young, tiller, mature-preharvest) were analysed. There were marked seasonal variations in abundances of these organisms, but the abundance pattern in various rice cultivation phases was comparable among rice growing seasons. The rice field was dominated by tubificids, baetids, chironomids and ceratopogonids. High populations of these organisms were observed during the plough, transplanting-young, and tiller phases, but lower in the other rice cultivation phases. The proliferation of larval *Agriocnemis femina* (Odonata: Zygoptera), one of the rice pest predators in the adult stage, was dependent on its interactions with 14 aquatic taxa in different phases; nine taxa in the plough phase and three to five taxa in other phases. Hemipterans, coleopterans, other odonates, chironomids, baetids and tubificids were important for successful emergence of *Agriocnemis femina* to a predatory adult. Rice cultivation managements that focus on enhancing the population of *Agriocnemis femina* would contribute to more effective biological control of rice pests in MRRF.

Key words: ***Aquatic invertebrates, benthic interactions, damselfly, rice cultlivation phases, rice field ecosystem, Sumatera.***

Handling Editor: ***Jennifer L. McCarthy***

Introduction

Tropical rice fields are usually inhabited by a diverse fauna of aquatic organisms (Fernando 1993; Heckman 1974, 1979; Victor & Ruben 1999). Some flying adults of Hemiptera (aquatic bugs), Coleoptera (beetles), and Odonata (dragonflies and damselflies) fly into the area and colonize the rice fields (Che Salmah *et al.* 2000; Fernando 1960; Leitao *et al.* 2007; Pires *et al.* 2015; Suhling *et al.* 2000); while other species spend their larval phase in the moist mud, growing rapidly in the aquatic medium and emerging as adults (Leitao *et al.* 2007; Pires *et al.* 2015). Rice cultivation activities within a short rice growing season regulate the abundance and diversity of these organisms (Asghar 2010; Che Salmah & Abu Hassan 2002; Hayasaka *et al.* 2012; Mogi 2007), and therefore,

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rice fields are colonized by organisms with short life cycles that are well adapted to the temporary nature of the rice field habitat (Heiss *et al.* 1986).

Rice fields also host a vast number of zooplankton and other aquatic invertebrates, including rice pests such as some species of Chironomidae (non-biting midges) and Ephydridae (shore flies) (Ali 1990; Simpson & Roger 1995). These also serve as a food source for fish during the inundated phase (Heckman 1979; Williams & Coad 1979). Adult Odonata, Ephemeroptera (mayflies), and various Diptera (flies) lay eggs immediately after the water is available, and their aquatic larval stages inhabit the benthos (Che Salmah 1996; Fernando 1993). In a short time these aquatic organisms are abundant in the rice fields (Amir Shah Ruddin *et al.* 2008; Hidaka 1998; Molozze *et al.* 2007; Suhling *et al.* 2000).

Rice cultivation is separated into phases following specific characteristics of the field during the cultivation process (Fernando 1993), and the composition of aquatic organisms has been shown to vary in different phases (Che Salmah *et al.* 1998). Hydrophilids, ostracods, gastropods and corixids succinate on ratoons of rice plants (*Oryza sativa* L.) during the fallow phase immediately after rice grains are harvested (Schoenly *et al.* 1998). Aquatic organisms start to colonize the rice field soon after the fallow field is ploughed and inundated. Many species of diving beetles (Dysticidae) and Odonata are more abundant in tiller and mature phases during which rice plants are actively producing plantlets and bearing grains (Che Salmah & Abu Hassan 2002; Mogi & Miyagi 1990). Changes in the abundance of aquatic organisms in relation to rice growth phenology have been observed in Japanese (Ban & Kiritani 1980), Malaysian (Amir Shah Ruddin *et al.* 2008; Che Salmah & Abu Hassan 2002), and Brazilian (Molozze *et al.* 2007) rice fields.

Odonata species are one of the main predators in rice fields during the aquatic larval stage (IRRI 2013; Leitao *et al*. 2007; Yamazaki *et al*. 2003), and more importantly in the adult stage (Ghahari *et al.* 2009; Martinez & Rodriquez-Castro 2007; Norela *et al*. 2013; Prasad 2010). In the rice field ecosytem, the abundance of larval dragonflies (Zygoptera and Anisoptera) has shown a strong relationships with the availability of their prey organisms (Bambaradeniya *et al.* 2004; Baumart & Santos 2010; Mogi 2007; Varela & Gaput 2013). Odonata larvae prey on a variety of small organisms up to the size of the tadpoles and small fish (Duxbury *et al.* 2010). During adult stage, the Zygoptera are important predators of rice pests particularly the plant and leaf hoppers and are effective biological control agents for these pests (Heong & Hardy 2009).

Simalungun district is one of the main rice producing areas in North Sumatera, Indonesia, covering an area of 4,386.6 km^2 with an estimated human population of 830,986 in 2012 (BPSPSU 2014). Rice farming is one of the main agricultural activities. Several rice varieties (Ciherang, IR36, IR42, IR 64, IR 66) are grown twice yearly in lowland, hilly and terraced rice fields located at altitudes ranging from 20 m a.s.l. to 1400 m a.s.l. Ecological studies of aquatic organisms in Indonesian rice fields are lacking, especially in the context of their roles in aquatic food web, and their contribution to the biological control of rice pests (Ansori 2000; Aswari 2013). Our observations and preliminary sampling efforts in 2008 (unpublished data), had indicated that a zygopteran species, *Agriocnemis femina* (Rambur), dominated the odonate larval and adult populations in the rice field of Manik Rambung in Simalungun district.

Taking into account the importance of aquatic organisms in rice fields, the present study was undertaken to investigate the community assemblages of aquatic organisms in MRRF during different rice growing seasons and rice cultivation phases. We anticipated that the assemblages of these organisms would be affected by seasonal variation and different cultivations phases due to dissimilarity of physical habitats and micro-environments. Based on the non-seasonal abundance pattern of rice field invertebrates reported by Che Salmah (1996), we predicted that the pattern of invertebrate richness in various cultivation phases in Manik Rambung rice field (MRRF) was not affected by rice growing season. We also investigated the interaction between *Agriocnemis femina* and other aquatic organisms which subsequently determined its successful emergence to a predatory adult that fed on several pest species of rice plant. We expected that this species would consume prey items based on their availability in the habitat.

Materials and Methods

This study was carried out at MRRF in the district of Simalungun in North Sumatera. Samples were collected at three sampling sites in the study area (Fig. 1). The area is located about 90 km from Medan City, the capital of Sumatera at 594–620 m asl between the latitude of

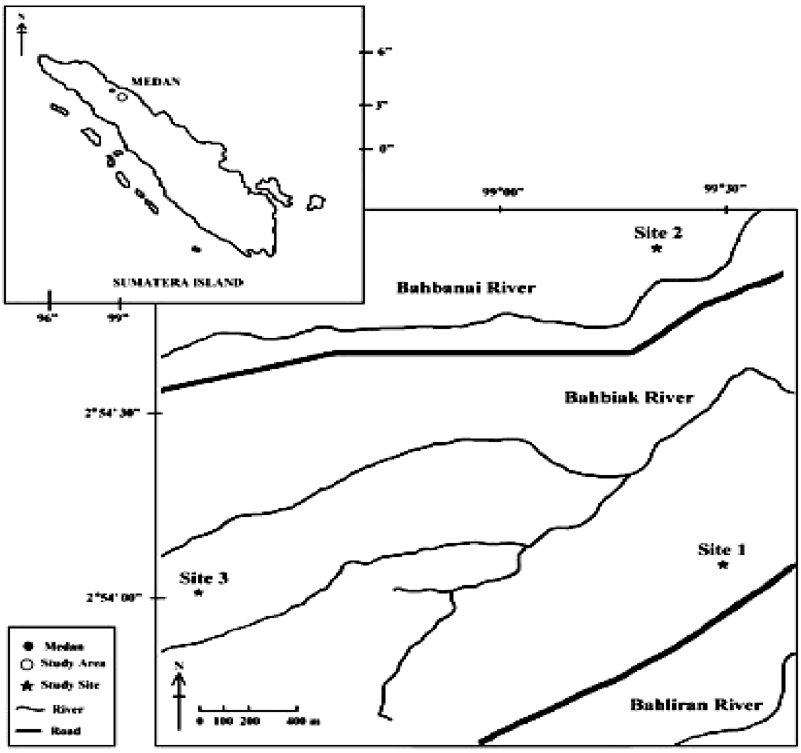

Fig. 1. Location of sampling sites in Manik Rambung rice field in north Sumatera.

2°53'52.8"N and 2°53'60.2"N, and the longitude of 98°00'24.4"E to 99°00'24.4"E.

In this district, two crops of rice were planted each year. Cultivation practices followed recommendations by the local agricultural department. Fertilizers were mechanically applied twice yearly. Herbicides (Ally-XP® and Rhodiamine®) and insecticide (Imidacloprid®) were minimally used to control weeds and rice pests respectively. We defined five rice cultivation phases in MRRF following the descriptions of Mogi & Miyagi (1990) and Stenert *et al.* (2009). They were categorized into fallow, plough, transplanting-young, tiller and mature-prehavest phases based on the water level in the field, the amount of surface water shaded by the rice plants, as well as the physical condition of the field. Fallow phase (F) is defined as uncultivated land area and starts immediately after harvest until the next rice growing season which may last up to two months. In the first part of the phase, the dry field is filled with uniform height rice stumps. Then rice ratoons and aquatic weeds start to grow in the fields which slowly accumulate from 3 cm to 5 cm of water. The substrate in plough phase (P) is slightly undulated due to the ploughing process and the tyre tracts left by the tractors. Soil depressions contain up to 40 cm of water which remains for 6 to 8 weeks. The transplanting-young field phase (TY) has transplanted rice plants, approximately 30 cm high. The water level may reach up to 10–20 cm. Rice plants in tiller phase (T) have completed tillering but have not eared, growing in less than 10 cm of water. The water surface is half-shaded by the plants. The last phase is the mature-preharvest phase (MP) which begins when the plants ear. It continues through the plants bearing flower panicles and developing grains, ending with the yellowing of the grains. The water depth is less than 5 cm and rice leaves have completely shaded the water surface.

Water quality

On each sampling occasion, measurements of physico-chemical parameters, such as water pH, water temperature and dissolved oxygen (DO) were made *in situ* at three randomly selected locations at each sampling site. Dissolved oxygen and water temperature were measured with a YSI-57 meter (YSI Inc., Yellow Springs, Ohio), whilst water pH was measured with a Termo-Orion

Model 210 pH meter. The measurements of water depth and plant height were taken using a metal measuring tape.

To analyze selected chemical parameters of the water, three separate water samples from each site were randomly collected into acid-washed (HCl 0.1N) 500-ml plastic bottles. Each appropriately labeled bottle was thoroughly rinsed with the rice field water immediately prior to collecting a sample. All water samples were transported to the laboratory in an ice chest, and kept at 4 ºC until analyzed. The ammoniacal-N, nitrate-N, and phosphate contents of the water were measured at appropriate wavelength using a YSI 9100 photometer test kit. Distilled water was used as a blank.

Sampling of aquatic organisms

Aquatic organisms were sampled from the three sites fortnightly from August 2008 to August 2010, covering four rice growing seasons. To gain a representative sample of the population of aquatic organisms in the area, thirty samples, which produced the standard error of less than 20%, were collected from the rice field (Elliot 1973).

Two samplers were used to collect the aquatic organisms from the rice field. The first was a core sampler (85 cm high, area = 1661 cm^2) which was pressed into the sediment and the substrate strirred by hand for two to three minutes (Wilson *et al.* 2008). The contents were collected and passed through a fish net (0.4 mm mesh) using a plastic dipper. The second sampler was a small aquatic net (14 × 14 cm, 0.4 mm mesh), which collected aquatic organisms between rice hills (Che Salmah 1996). It was dragged on the sediment over a distance of approximately a meter. Equal number of samples were collected using each sampler to make up the total of 30 samples during each sampling occasion. The collected organisms were transferred into a plastic bag and placed in a chilled ice box to reduce predation and maintain samples in a good condition during transportation. All samples were processed at the laboratory of Agroecotechnology Department, Universitas Sumatera Utara in Medan.

In the laboratory bigger aquatic organisms were separated from the sample by washing the samples in a tray and screened through 1 mm mesh sieve. The smaller organisms in the debris that passed through the sieve were further collected in 0.4 mm mesh sieve. All organisms were sorted visually and preserved in 75% ethanol. They were identified to respective taxa or species under a dissecting microscope (Olympus CX41, Olympus Tokyo, Japan) using keys and descriptions (Edmonson 1992; Merritt *et al.* 2006; Morse *et al.* 1994; Orr 2005; Usinger 1956).

Data analysis

Variations in mean abundance of aquatic organisms were analyzed using Kruskal-Wallis test (at $P < 0.05$) for non-normally distributed data to determine differences among sampling times, rice growing seasons, and rice cultivation phases. The corelation between *Agriocnemis femina* with aquatic organisms was investigated using Spearman Correlation Analysis. To produce a generalized model from a large number of potential explanatory variables which were collected over a large time interval (2 years), the stepwise multiple regression was run on abundance data of *Agriocnemis femina* against other aquatic organisms collected during four rice growing seasons. In addition, multiple regression analyses were performed to predict the dependence of *Agriocnemis femina* on other aquatic organisms as possible food source, or as its predators at various phases of rice cultivation. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows®.

The ecological indices, such as Shannon-Wiener, Simpson, Menhinick, Margaleff, Pileou, Jaccard and Dominance Index (Rohlf & Sokal 1973) were calculated to compare the community pattern of aquatic organisms during various seasons of rice cultivation in MRRF.

Results

Water quality

The water quality at the three sampling sites did not vary significantly, although some parameters differed slightly at certain rice growing phases. This may be due to rice cultivation activities such as the application of fertilizers and pesticides. Water depth at all sites was between 14.76 cm and 24.39 cm, and water pH varied from slightly acidic to neutral (5.97–7.25). Water temperature fluctuated very little (26 °C to 26.70 °C) over the growing season. Dissolved oxygen (DO) was slightly high (6.07–6.32 mg l^{-1}) while biochemical oxygen demand (BOD) fell between 2.48 and 2.5 mg l^{-1}. Nitrate contents in the water fluctuated between 6.47 mg l^{-1} and 11.11 mg l^{-1}. Phosphate and ammoniacal-N levels were 4.36 mg l^{-1} to 6.69 mg l^{-1} and 0.07 mg l^{-1} to 0.09 mg l^{-1}, respectively.

Abundance of aquatic insects

In the course of study over four rice growing seasons, 48,127 aquatic invertebrates were collected to represent 6 orders, 21 families and 25 species (Table 1). Oligochaeta, Ephemeroptera, Hemiptera and Diptera were only identified to the family level due to the lack of suitable keys. The families of Odonata and Coleoptera were identified to genera and species when possible. Three taxa (Oligochaeta, Ephemeroptera and Diptera) were widely distributed in the study sites but the orders Coleoptera and Hemiptera were less abundant. Baetidae (27.97%), Chironomidae (27.40%) and Tubificidae (22.91%) represented the highest percentage of aquatic organisms, while *Ictinogomphus decorates* (Selys) (0.01%) and *Potamarcha congener* (Rambur) (0.01%) were the lowest (Table 1). According to Kandibane *et al.* (2005) the percentage Dominance Index of aquatic organisms in general varied from 0.01% to 27.97%. Using the scores, Oliveira and Vasconcelos (2010) divided the Dominance Index (DI) into three categories; D = Dominant (≥ 5% individual); A = Accessory (2.5% ≤ D ≤ 5% individual) and O = Occasional (< 2.5% individual). Following this categorization, Baetidae and Tubificidae were dominant, and Coenagrionidae was accesory in the study area, while other families were all classified as occasional invertebrates. Abundances of aquatic invertebrates were significantly different during the time of sampling ($\chi^2 = 1274.84$, $df = 47$, $P < 0.001$) and rice growing seasons ($\chi^2 = 16.393$, $df = 3$, $P = 0.001$).

Separating the collection according to rice cultivation phenology, the highest number of aquatic invertebrates was collected during the plough phase, then transplanting-young phase, followed by the tiller phase. Very few aquatic organisms were collected during the fallow and mature-preharvest phases. Statistically, their abundances were significantly different (Mann-Whitney U test, $P < 0.05$) during various phases of rice cultivation ($\chi^2 = 8.618$, $df = 4$, $P = 0.004$).

Variations in the abundance of all invertebrates in the four seasons and various cultivation phases in MRRF can be attributed to the differences in distribution of the dominant families; Tubificidae, Baetidae, Chironomidae and Ceratopogonidae (Table 2). Baetids showed decreasing abundance from season one to season four which was fairly similar to the tubificids. Chironomids and ceratopogonids shared identical distribution pattern with their highest abundances recorded in season two. All families occurred at the lowest abundance in season four compared to other seasons. Among the cultivation phases, very high abundance of these families were observed during the plough phase, then in transplanting-young phase but much fewer organisms in the fallow, tiller and mature-preharvest phases. Invertebrate diversity were relatively high (H' = 2.99, 1–D = 0.78), as well as their richness scores (R1 and R2) (Table 3). Variations among taxa abundances (evenness) and similarity in community structure were relatively high.

Interaction among aquatic organisms

The role (prey and predator) of aquatic organisms collected from MRRF were referred to available literatures (Baumart & Santos 2010; Litsinger *et al.* 1994; Mogi 2007; Pritchard 1964; Simpson *et al.* 1994; Yamazaki *et al.* 2003). Four families (Lumbricidae, Tubificidae, Baetidae and Caenidae) were found to be prey, and four other families (Belostomatidae, Corixidae, Nepidae, Pleidae, and Dytiscidae) were classified as predators. Eight families (Mesoveliidae, Notonectidae, Ceratopogonidae, Noteridae, Chironomidae, Veliidae, Culicidae and Tipulidae) and other taxa were considered as either prey or prey/predator.

The relationships of *Agriocnemis femina* with other aquatic organisms in different rice cultivation phases was tabulated (Table 4). Relatively strong correlations between *Agriocnemis femina* with *Ischnura senegalensis* (Rambur) were detected during two cultivation phases (P, $\rho = 0.590$; MP, $\rho = 0.490$, $P < 0.01$), followed by *Orthetrum sabina* (F, $\rho = -0.437$; P, $\rho = 0.754$), *Pantala flavescens* (Fabricius) (T, $\rho = -0.515$; TY, $\rho = -0.453$; M, $\rho = 0.545$), and Notonectidae (F, $\rho = -0.427$; P, $\rho = 0.780$, $P < 0.01$). This could imply that they were either important prey or predators for the *Agriocnemis femina*. Hydrophilidae and Chironomidae were strongly correlated ($P < 0.05$) during fallow, transplant- young and tiller phases, while tipulids were more significant during the three later phases of rice cultivations. Corixidae, Hydrophilidae and Noteridae were important to *Agriocnemis femina* at least in one of the cultivation phases.

Table 1. Composition of aquatic organisms collected in four rice growing seasons (2008–2010) in MRRF. DI = Dominance Index; D = Dominant (≥ 5%); A = Accessory (2.5% ≤ D ≤ 5%); O = Occasional (< 2.5%).

Class/Order/Family/Species	Total organisms	DI (%)	Categories of DI
Oligochaeta			
Lumbricidae	186	0.39	O
Tubificidae	11028	22.91	D
Ephemeroptera			
Baetidae	13464	27.97	D
Caenidae	235	0.49	O
Odonata			
Zygoptera			
Coenagrionidae	1373	2.86	A
Agriocnemis femina (Rambur)	776	1.61	
Agriocnemis pygmaea (Rambur)	339	0.70	
Agriocnemis rubescens (Selys)	39	0.08	
Ischnura senegalensis (Rambur)	147	0.31	
Pseudagrion microcephalum (Rambur)	35	0.07	
Pseudagrion pruniosum (Burmeister)	17	0.04	
Pseudagrion rubriceps (Selys)	20	0.04	
Anisoptera			
Gomphidae	5	0.01	O
Ictinogomphus decorates (Selys)	5	0.01	
Libellulidae		2.49	O
Acisoma panorpoides (Rambur)	16	0.03	
Crocothemis servilia (Drury)	89	0.18	
Diplacodes trivialis (Rambur)	10	0.02	
Neurothemis ramburii (Kaup in Brauer)	20	0.04	
Neurothemis terminata (Ris)	34	0.07	
Orthetrum sabina (Drury)	578	1.20	
Orthetrum testaceum (Burmeister)	26	0.05	
Pantala flavescens (Fabricius)	357	0.74	
Potamarcha congener (Rambur)	6	0.01	
Trithemis aurora (Burmeister)	28	0.06	
Tholymis tillarga (Fabricius)	30	0.06	
Hemiptera			
Belostomatidae	36	0.49	O
Corixidae	248	0.52	O
Mesovellidae	299	0.62	O
Nepidae	161	0.33	O
Notonectidae	226	0.47	O
Veliidae	285	0.59	O
Pleidae	241	0.50	O
Coleoptera			
Dysticidae	162	0.33	O
Laccophilus sp.	90	0.19	
Cybister sp.	72	0.15	
Hydrophilidae		0.53	O
Berosus sp.	255	0.53	
Noteridae		0.54	O
Noterus sp.	259	0.54	
Diptera			
Chironomidae	13188	27.40	D
Ceratopogonidae	4332	9.00	D
Culicidae	471	0.98	O
Culex sp.	253	0.53	
Anopheles sp.	218	0.45	
Tipulidae	279	0.58	O

Table 2. Dominant families of aquatic organisms from four rice growing seasons. RGS = Rice Growing Season. F = Fallow phase, P = Plough phase, TY = Transplanting-Young phase; T = Tiller phase; MP = Mature-Preharvest phase.

Aquatic organism	RGS	Cultivation phases					
		F	P	TY	T	MP	Total
Tubificidae	1	14	2101	1020	24	19	3178
	2	10	1636	976	19	18	2659
	3	16	1850	840	21	16	2743
	4	14	1536	859	20	19	2448
Baetidae	1	0	3195	895	14	10	4114
	2	0	2492	1092	20	9	3613
	3	0	2264	967	17	9	3257
	4	0	1771	682	19	8	2480
Chironomidae	1	5	2316	908	12	5	3246
	2	10	2620	1015	12	12	3670
	3	8	2622	962	13	7	3612
	4	3	1861	795	11	7	2677
Ceratopogonidae	1	3	664	315	22	16	1020
	2	6	656	526	19	13	1220
	3	4	636	420	23	15	1098
	4	3	570	381	18	12	984

Table 3. Community features of aquatic organisms in MRRF as indicated by the scores of ecological indices.

Ecological indices	Values
Shannon-Wiener Index (H')	2.99
Simpson Index (1 – D)	0.78
Menhinick Index (R1)	2.85
Margaleff Index (R2)	1.14
Pielou Index (E)	0.43
Jaccard Index (Cj)	0.86

Table 4. Relationships of *Agriocnemis femina* with aquatic organisms (Spearman's Correlation Analysis) in each rice cultivation phase (* = $P < 0.05$ and ** = $P < 0.01$). F = Fallow phase, P = Plough phase, TY = Transplanting-Young phase; T = Tiller phase; MP = Mature-Preharvest phase.

Family/ species	F	P	TY	T	MP
I. senegalensis	0.266	0.590**	0.160	0.373	0.490*
O. sabina	–0.437*	0.754**	–0.347	–0.218	–0.192
P. flavescens	0.187	0.209	–0.515**	–0.453*	0.545**
Corixidae	–0.034	0.757**	–0.257	0.029	0.048
Mesoveliidae	–0.280	–0.117	–0.014	–0.057	–0.685**
Notonectidae	–0.427*	0.780**	0.243	0.063	0.232
Other Hemiptera	–0.115	0.405*	0.046	0.046	0.264
Hydrophilidae	0.610**	0.732**	–0.293	–0.293	0.009
Noteridae	0.013	0.519**	0.243	0.243	0.221
Chironomidae	0.763**	0.242	–0.537**	–0.501*	–0.794
Anopheles sp.	0.329	0.489*	–0.236	–0.141	0.082
Tipulidae	0.104	0.010	–0.408*	–0.396*	0.527**

A regression equation represents a good prediction of the relationships between *Agriocnemis femina* and various aquatic organisms. The result of stepwise multiple regression analysis between *Agriocnemis femina* and other aquatic organisms during four rice growing seasons indicated the abundance of *Agriocnemis femina* in MRRF was regulated by 14 aquatic organisms: Baetide, Belostomatidae, Lumbricidae, Ceratopogonidae, *Agriocnemis pygmaea* (Rambur), *Orthetrum sabina* (Drury), Veliidae, *Pseudagrion pruniosum* (Burmeister), *Potamarcha congener* (Rambur), *Anopheles* sp., *Trithemis aurora* (Burmeister) Tipulidae, *Crocothemis servilea* (Drury) and Nepidae ($R^2 = 0.655$, $F = 190.59$, $df = 14{,}39$, $P = 0.00$). Relationship of *Agriocnemis femina* with its potential preys and predators in different rice cultivation phases are expressed by regression models (Table 5). During the fallow phase, the dragonfly *Orthetrum sabina* and three other insect families (Notonectidae, Hydrophilidae and Chironomidae) were important predators of *Agriocnemis femina*. In the plough phase, two species of Odonata (*Ischnura senegalensis* and *Orthetrum sabina*), Diptera *Anopheles* sp., Lumbricidae, Corixidae, Notonectidae, Hydrohilidae, Noteridae, and other Hemiptera regulated the abundance of *Agriocnemis femina*.

Only four families (Tubificidae, Baetidae, Chironomidae, Tipulidae) were prey and the odonate *Pantala flavescens* was the predator of *Agriocnemis femina* in transplant-young phase. Then in tiller phase, Baetidae, Chironomidae and Tipulidae remained as an important food source for *Agriocnemis femina*. Finally, odonates *Ischnura senegalensis* and *Pantala flavescens* with ephemeropteran Baetidae determined the abundance of *Agriocnemis femina* in mature-preharvest phase.

Discussion

Abundance of aquatic organisms

A large collection of aquatic invertebrates over a considerable period of time represented a reliable assemblage of these organisms in MRRF. Comparing to other studies, the diversity of invertebrates in MRRF was much lower than communities recorded from rice fields in Japan (44 taxa, Yamazaki *et al.* 2003), Portugal (71 taxa, Leitao *et al.* 2007), and Italy (173 taxa, Lupi *et al.* 2013). Difficulty in identification was the main

Table 5. Summary of multiple regression models of *Agriocnemis femina* and other aquatic invertebrates in phases of rice cultivation.

Phase	*df*	R^2	F	*P* values	Model
Fallow	19	0.755	14.67	0.000	Y = 0.356 (*O. sabina*) + 0.317 (Notonectidae) + 0.368 (Hydrophilidae) + 0.236 (Chironomidae) – 0.97
Plough	14	0.846	8.51	0.000	Y = 1.159 (Lumbricidae) + 0.048 (*I. senegalensis)* + 0.242 *(Orthetrum sabina)* + 1.447 (Corixidae) + 0. 664 (Notonectidae) + 0.123 (Other Hemiptera) + 0.439 (Hydrophilidae) – 0.576 (Noteridae) – 0.125 (*Anopheles* sp.) – 1.518
Transplant to Young	18	0.627	10.24	0.002	Y = 0.142 (Tubificidae) – 0.089 (Baetidae) – 0.419 (*P. flavescens*) – 0.658 (Chironomidae) – 1.397 (Tipulidae) + 9.696
Tiller	20	0.466	12.69	0.005	Y = 0.139 (Baetidae) – 0.589 (Chironomidae) – 1.407 (Tipulidae) + 7.815
Mature to Preharvest	20	0.617	40.57	0.000	Y = 3.998 (Baetidae) + 1.116 (*Ischnura senegalensis*) – 0.192 (*P. flavescens*) – 0.912

reason for this low record because 12 of the families collected including the most abundant (Lumbricidae and Tipulidae) were not identified to genus or species. However the communities in MRRF were comparable to studies in Malaysia (39 taxa, Lim 1992; 21 families, Maimon *et al.* 1994); and higher than was recorded in the Philippines (9 taxa, Banwa *et al.* 2013), France (23 species, Suhling *et al.* 2000), India (26 taxa, Roger *et al.* 1991), and Brazil (28 taxa, Mollozze *et al.* 2007). MRRF scored a higher diversity index of aquatic organisms compared to rice fields of Southern Mexico (Bond *et al.* 2006) and Southern India (H' = 1.74–2.44, Gaurav *et al.* 2007); but slightly lower than rice fields in Punjab Shilvalik, India (H' = 2.98–3.02, Gaurav *et al.,* 2007), and Tamil Nadu, India (H' = 2.82 – 3.33, Anbalagan *et al.* 2013).

Diptera, Ephemeroptera and Oligochaeta occurred in high numbers at the study sites in all rice growing seasons. Chironomids (Diptera) were the most commonly collected organisms, followed by baetids (Ephemeroptera) and tubificids (Oligochaeta). Likewise, a high abundance of Chironomidae has been reported from conventionally managed rice fields in France (Mesleard *et al.* 2005). The dominance of Oligochaeta, Ephemeroptera and Diptera was similarly observed by Stenert *et al.* (2009) in wetlands of southern Brazil and in disturbed riverine system in India (Bahuguna *et al.* 2004). A few odonate species (*Ictinogomphus decoratus*, *Potamarcha congener*, *Orthetrum sabina, Pantala flavescens, Diplacodes trivialis* (Rambur), *Agriocnemis femina* and *Agriocnemis pygmaea*) belonged to the lower assemblage of aquatic invertebrate in MRRF. In this group, *Orthetrum sabina, Pantala flavescens*, *Agriocnemis femina and Agriocnemis pygmea* had relatively high abundances compared to other species. Odonate larvae are voracious predators that consume prey which include: oligochaetes, ephemeropterans, Baetidae, Caenidae and dipteran Chironomidae (Baker *et al.* 1999; Katayama 2013; Leitao *et al.* 2007; Yamazaki *et al.* 2003). McDonald & Buchanan (1981) and Mogi & Miyagi (1990) reported that Odonata larvae also consumed larvae of mosquito species that acted as vectors of human pathogens. However, they found that the odonates did not usually share the same niche with their mosquito prey in the rice field.

The richness pattern of aquatic organisms in MRRF rice fields was comparable in all four seasons, with strikingly high numbers in the plough phase, but lower numbers during the fallow and mature-preharvest phases. Differences in macroinvertebrate abundance among rice phases were also reported by Asghar (2010) and Stenert *et al.* (2009), who recorded higher density and richness of macroinvertebrates during the flooded phases (plough, tiller) and lower densities during the fallow phase.

High abundances of Oligochaeta, Baetidae, *Agriocnemis femina, Agriocnemis pygmaea, Orthetrum sabina, Pantala flavescens,* Chironomidae and Ceratopogonidae from the plough to tiller phases have been connected to ample water supply from proper irrigation (Mogi 1993). The annelids, dipterans and ephemeropterans are feeding on the rich detritus from soft muddy substrate especially during the plough phase, while other insects such as coleopterans, hemipterans, odonate are either prey or predators (Asghar 2010; Hayasaka *et al.* 2012; Merritt & Cummins 1984). Odonates *Ishnura elegans* (Vander Linden) (Heads 1985); *Neurothemis tullia* (Rambur) (Che Salmah 1996); *Enalagma boreale* (Selys) (McPeek & Peckarsky 1998); *Sympetrum frequens* (Selys) (Mogi 2007); and *Crocothemis servilea* (Drury) (Varela & Gaput 2013) live on insects such as baetids, corixids, notonectids, hyrophilids, *Culex tarsalis* L. (mosquito) and *Aedes aegyptii* L. (Al-Shami *et al.* 2010; Lupi *et al.* 2013; Mogi 2007), which some of them occurred in high abundances in various rice cultivation phases in MRRF.

In areas suffering irregular water supply, the abundance and diversity of aquatic organisms are easily affected because many species have no adaptation to tolerate or escape the dry phase (Chovanec & Waringer 2001; Wissinger 1999). Available ditches or ponds in vicinity of rice fields may provide some water for the growth of aquatic organisms during the dry period. However, very low water levels in MRRF during the mature phase (less than 5 cm) and a completely shaded water surface prior to grain harvesting, reduced the abundance of aquatic organisms such as Tubificidae, Baetidae, Hydrophilidae, Chironomidae, Ceratopogonidae, *Orthetrum sabina* and *Pantala flavescens*. Other studies reported that the abundance and diversity of aquatic organisms in rice fields is also regulated by variations in habitat suitability, availability of food sources, soil drainage, machinery use and herbicide application (Asghar 2010; Bambaradeniya 2000; Leitao *et al.* 2007; Wissingger 1988).

Negative effects of pesticides on aquatic organisms in rice fields have been previously documented by various authors (Schoenly *et al.* 1998; Suhling *et al.* 2000; Wilson *et al.* 2008). The application of chemicals such as fertilizers, herbicides and insecticides often leads to nutrient enrichment of surface waters (Baumart & Santos 2010; Dudgeon 2000; Jergentz *et al.* 2005), as observed in MRRF after the application of insecticides during the end of transplant-young phase and beginning of tiller phase. Consequently, the abundance of aquatic organisms, particularly tubificids, baetids and chironomids, were much reduced at these times.

Rice fields are artificial habitats that accommodate various plant and animal species (Cabral *et al.* 1998). During low water level (3–5 cm), or the completely dry fallow phase in MRRF, dense growth of hygrophilous weeds such as *Cyprus* sp., *Eleocharis* sp., *Kylingga* sp., *Ludwigia* sp., *Monochoria* sp., and *Sagittaria* sp. might reduce the availability of habitat for aquatic organisms, as well as the predation efficiency of various predators. The foraging efficiency of predators determines the distribution of prey and predators in rice fields (Leitao *et al.* 2007; Maimon *et al.* 1994; Schoenly *et al.* 1998). However, the application of herbicides to control weeds during this phase could result in a positive effect to the odonates but detrimental to some aquatic organisms (Che Salmah *et al.* 2012). Steinert *et al.* (2009) reported that the herbicide glyphosate which effectively kills sedges and grasses, was highly toxic to ephemeropterans, coleopterans and dipterans.

Interaction among aquatic organisms

The abundance of food resources in rice fields results in the proliferation of prey species, which eventually leads to an increase in predator abundance. Thorp & Cothran (1984) reported that the density of macroinvertebrates such as chironomids was positively correlated with the densities of their predatory odonates. In MRRF, six taxa of aquatic organisms inhabited this ecosystem, and most of them showed positive or negative interactions with the others. Hemiptera, Odonata and Coleoptera are exclusively predatory although they may be considered prey at young stages. Several co-existing odonates could reverse their prey-predator role depending on their sizes, because usually this relationship was size specific (Conrad *et al.* 2002; Wissinger 1988). Odonates are multivoltine with overlapping generations in tropical rice fields (Che Salmah *et al.* 1998). Bigger larvae of *Crocothemis servilea, Orthetrum sabina* and *Pantala flavescens* are reported to consume smaller sized damselflies (*Agriocnemis pygmaea* and *Ischnura senegalensis*) and dragonflies (Kandibane *et al.* 2005; Krishnasamy *et al.* 1984; Varela & Gaput 2013).

The results from this study indicated strong interactions of *Agriocnemis femina* with three species of Odonata; *Ischnura senegalensis* (during the plough and mature-preharvest phases), *Orthetrum sabina* (in the fallow and plough phases) and *Pantala flavescens* (during the transplant-young, tiller and mature-preharvest phases). *Agriocnemis femina* is a predator in the larval stage which is completely aquatic. During its terrestrial adult stage, *Agriocnemis femina* is an important predator of rice pests, especially the leafhoppers (e.g. *Nephotettix* sp.) and planthoppers (e.g. *Nilaparvata* sp.) (Heong & Hardy 2009; Krishnasamy *et al.* 1984; Litsinger *et al.* 1994). The results of the present study showed that this species was the most dominant zygopteran larvae in MRRF. During the larval stage, *Agriocnemis femina* showed both positive and negative interactions with many taxa of aquatic organisms which suggested that these organisms were important for its survival in this rice field.

In the four rice growing seasons, the abundance of *Agriocnemis femina* was positively dependent on 14 taxa of aquatic organisms (Baetide, Belostomatidae, Lumbricidae, Ceratopogonidae, *Agriocnemis pygmaea, Orthetrum sabina,* Veliidae, *Pseudagrion pruniosum*, *Potamarcha congener, Anopheles* sp., *Trithemis aurora*, *Crocothemis servilea* and Nepidae). With the exception of Lumbricidae and Ceratopogonidae, most of these taxa are predators of *Agriocnemis femina* at least at certain periods of their life cycle. Nevertheless, some predators such as: *Agriocnemis pygmaea, Orthetrum sabina,* Veliidae, *Pseudagrion pruniosum, Potamarcha congener, Trithemis aurora and Crocothemis servilea,* could become prey when their sizes were suitable to be eaten by *Agriocnemis femina* whilst Lumbricidae, Ceratopogonidae and Tipulidae were definitely its preys. This finding is in line with other studies which indicate that in a diverse ecosystem, a predator species tends to consume a wide variety of prey items. For example, Cabral *et al.* (1998) had identified 12 prey taxa in the stomach of a mosquito fish, *Gambusia holbrooki* (Girard) within a rice growing season.

Agriocnemis femina interacted with the same taxa of aquatic organisms in more than one rice cultivation phases. For instance, Chironomidae was consumed by this odonate larva in fallow, transplant-young and tiller phases, while Baetidae was an important food source in transplant-young, tiller and mature phases. Three dragonfly species along with Tipulidae, Hydrophilidae, and Notonectidae interacted with *Agriocnemis femina* in two cultivation phases. Prey preference can play an important role in the intake of prey species. Chironomids have been reported as a preferred food for *Agriocnemis femina* (Anna & Bradley 2007; Varela & Gaput 2013), as well as *Agriocnemis pygmaea* (Park *et al.* 1995), which supports its selection by *Agriocnemis femina* in many rice cultivation phases in the present study. Other prey organisms included oligochaetes (Schaffner & Bradley 1998), whilst ephemeropterans baetids and caenids, culicids, *dysticids*, hydrophilids and noterids were highly preferred by *Ischnura elegans* (Suhling *et al.* 2000).

In general, the availability of aquatic organisms in each cultivation phase affected the abundance of *Agriocnemis femina*. Limited availability of prey taxa such as Noteridae (plough phase), *Anopheles*, Baetidae, *P. flavescens* (transplant-young phase), Chironomidae, Tipulidae (tiller phase), shaped the abundances of some aquatic organisms, which indirectly influenced the abundance of *Agriocnemis femina*. Among all phases, the highest abundance of *Agriocnemis femina* (422 individuals) was collected in the plough phase. During this phase *Agriocnemis femina* interacted with nine taxa of aquatic organisms. A high water level (up to 40 cm) and ample decomposed organic matter, led to a high quantity and quality of larval food which was conducive to the proliferation of various organisms. Consequently, the highest number of invertebrates was collected during the plough phase, which subsequently contributed to the highest abundance of *Agriocnemis femina*.

As reported by the present study, variations in the abundances of aquatic organisms following the changes in water level during the submerged phases of rice cultivation (plough, transplanting-young and tiller phases) have been documented by Che Salmah *et al.* (1998) and Pereira *et al.* (2000). Accordingly, the richness of prey at the particular time in the habitat is reflected by the diversity of prey species taken by a predator (Bambaradeniya *et al.* 1998; Lima 2002; Sih *et al.* 1998). Such association was also observed by Molozze *et al.* (2007) in Brazilian rice fields. In MRRF, low water level (fallow, transplant-young and mature-preharvest phases), high amount of canopy (mature phase) and pesticides applications (during transplanting-young, tiller phases) were among other factors that negatively influenced the abundances of prey and predators. During these phases fewer organisms were available to *Agriocnemis femina* which was translated into its lower abundances during these intervals.

The adverse effect of pesticides on Odonata, which includes reduced larval biomass as well as larval and adult population decreases, were thoroughly discussed by many authors (Roger *et al.* 1991; Simpson & Roger 1995; Suhling *et al.* 2000; Leitao *et al.* 2007). In MRRF, existing pesticide use was rather minimal. With a properly guided rice pest management programs (by the relevant authorities); such as application of less harmful insecticides only at the most suitable periods, and good water management; the population of *Agriocnemis femina* could be enhanced, thus increasing the potential of biological control of rice pests by this damselfly species.

Conclusion

Aquatic organisms occurred in fairly high diversity and abundances in different rice growing seasons and rice cultivations phases in MRRF, but the overall abundance pattern was very similar in all seasons. Aquatic organisms flourished in deeper water with high amounts of organic matter during the plough and transplanting-young phases. These organisms served as both a food resource and potential predators for *Agriocnemis femina*, a predator of rice pests during its adult stage. The abundance of *Agriocnemis femina* varied in different rice cultivation phases following the availability of its prey. In view of the great potential of adult *Agriocnemis femina* as a biological control agent of rice pests, management of MRRF focusing on enhancement of this species will contribute to the sustainability of rice cultivation in Simalungun district.

Acknowledgements

We are grateful to many people who helped us in the field and in the laboratory during the undertaking of this research. We are indebted to the farmers; Silalahi, Tarigan and Napitupulu who allowed us to work in their rice fields in Manik Rambung. We extend our gratitude to two anonymous reviewers whose comments and suggestions tremendously improved the final version of this manuscript. This project was funded by a research grant DIKTI RI 2008-2010 of the Ministry of Higher Education, Indonesia.

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(Received on 18.11.2014 and accepted after revisions on 22.10.2015)