International Journal of Academic Management Science Research (IJAMSR)
ISSN: 2643-900X
Vol. 9 Issue 4 April - 2025, Pages: 55-79

Influence of Application of Agrochemicals on the Benthic Macroinvertebrate Species Diversity and Abundance in Ahero Irrigation Scheme, Kisumu County, Kenya

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***Abstract**: Irrigated rice fields are considered temporary wetland agro-ecosystems managed with variable degree of intensity. They are dynamic environments with high biological diversity compared to other agro-ecosystems and are highly disturbed with routine rice farming practices such as ploughing, rotavation, puddling and application of agrochemicals. There is little information on the influence of these practices on benthic macroinvertebrates in lentic agro-ecosystems such as rice fields. Therefore, the main objective of this study was to establish the influence of application of agrochemicals on the benthic macro-invertebrate species abundance and diversity in Ahero Irrigation Scheme. The study adopted descriptive longitudinal research design. The benthic macroinvertebrate community were sampled using Ekman grab, washed through a 1 mm mesh sieve, fixed in 5% buffered formaldehyde, then sorted, identified and counted using a dissecting stereo microscope. Results indicated that total mean abundance for benthic macroinvertebrates varied significantly (One-Way ANOVA at $\alpha = 0.05$, $F_{(5,114)} = 100.440$, $p = 0.000$) during various operations in the rice fields. The highest value (245.60±40.674) was recorded immediately after fertilizer application at flowering stage and the lowest value (81.3±15.26) was recorded during land preparation. There was a moderate effect size ($Eta^2 = 0.183$ or after fertilizer application at flowering stage and the lowest mean value (81.3±15.26 18.3%) in plots A, B, D and G, as well as in plots K, L, M and N ($Eta^2 = 0.192$ or 19.2%). A total of 22,414 individuals distributed among 29 taxa were recorded for the two crop cycles. The most abundant macroinvertebrate orders were Hemiptera 30% (5,632), Diptera 28% (5,199), Coleoptera 27% (5,158), Ephemeroptera 9% (1,630) and Odonata 6% (1,157). Order Hemiptera had the highest families (6) while Ephemeroptera had the least (2). Chironomidae (2186 individuals) were the most abundant family present in all the sites since they were capable of living in condition of total oxygen depletion for some hours and present greatest environmental plasticity, living where other more sensible organisms are absent. High biodiversity indices were observed with Shannon Diversity (H') values ranging between 3.059 to 3.16, Pielou's evenness (J) ranged between 0.910 to 0.937 and taxon richness (S) ranged between 27 to 29. The findings for benthic macro invertebrates will be used to monitor ecological integrity of the rice agroecosystems.*

1. INTRODUCTION

Studies on rice farming have pointed out that chemical runoff from paddy fields are responsible for the contamination of aquatic agro-ecosystems (Nakano, Miyazaki, Yoshida, Ono, & Inoue, 2004) (Ahmad, Rashid, Ismail, & Mohamed, 2014). Earlier (Mesleard, Garnero, Beck, & Rosecchi, 2005) indicated that practices associated with crop management such as land preparation, use of herbicides, insecticides, fertilizers and irrigation methods, can modify the feeding pattern and alter the development of animal communities present in rice fields, especially the invertebrates decreasing the species richness. In their work (Faria, Nogueira, & Soares, 2007) reported that localities with high rates of contamination by pesticides and heavy metals, showed altered values of biotic factors different from contamination-free areas or areas with low contamination, and attributed this difference to the presence of chemicals in the environment. In Kenya little research has been done on the relationships between agricultural practices and benthic macro-invertebrate community structure in lentic ecosystem. A study done on biodiversity characteristics of small high-altitude tropical man-made reservoirs in the Eastern Rift Valley showed that benthic macro-invertebrate community abundance was not very diverse despite the accumulation of allochthonous detritus (Mwaura F., 2002). In a study (Rizo-Patron, 2013) found that changing physical-chemical conditions of paddy water can change the surrounding communities of benthic macroinvertebrates. Separate studies have related macro-invertebrate composition, density, diversity or assemblage to the aquatic environmental conditions (Ndaruga, Ndiritu, Gichuki, & Wamicha, 2004) (Masese, Raburu, & Muchiri, 2009) (Raburu, Masese, & Mulunda, 2009) (Mbaka, M'Erimba, Thiong'o, & Mathoko, 2014). These studies did not look at macroinvertebrate assemblages in rice agroecosystems. In Mwea (Onderi, 2016) assessed the suitability of effluents from irrigation scheme for re-use in rice irrigation so as to reduce the problems of water shortages and environmental degradation. This study however, concentrated on impact of nutrients on crops but failed to look at their effect on benthic macro-invertebrates. Further (Onderi, 2016) found that irrigation effluents were enriched with nutrients such as nitrates as they flow down the canals leading to eutrophication.

Some studies have revealed that the overuse of inorganic fertilizers to increase agricultural productivity in Kenya contributes to water pollution through runoff impacting on the aquatic life (Njuguna, Yan, Gituru, Wang, & Wang, 2017). For instance, the Kenyan side of Lake Victoria has the highest Biological Oxygen Demand (BOD) with atmospheric deposition and land runoff together accounting for 90% of P and 94% of N input into the lake (Scheren *et al*., 2020). Earlier on a study showed that when pollution goes beyond the self-purifying ability of aquatic ecosystems, death of aquatic animals starts following habitat destruction (Alavaisha, Lyon, & Lindborg, 2019). High nitrate values in rice irrigation schemes as a result of extensive use of inorganic fertilizers, affects the physical and chemical characteristics of the water leading to deterioration of the water quality and aquatic life (Njue, Magana, & Githae, 2023). Despite the fact that studies have attempted to relate macro-invertebrate composition, density, diversity or assemblage to environmental condition, the aspect of rice paddy agrochemical application has not been investigated in depth. The use of macro-invertebrate in environmental assessment and monitoring of the environmental quality in rice paddies is still uncommon in rice agrecosystem. This study therefore established the effect of application of agrochemicals in lentic waters on the benthic macroinvertebrate species diversity and abundance in rice paddies in Ahero Irrigation Scheme.

2. LITERATURE REVIEW

Globally, studies have shown that agricultural practices associated with the rice crop management such as irrigation and the application of pesticides and fertilizers affect the colonization of macroinvertebrates, decreasing the species richness (Suhling, et al., 2000) (Mesleard, Garnero, Beck, & Rosecchi, 2005). These practices can modify the overall health of the aquatic ecosystem through organic pollution and eutrophication (Yang, Wu, Hao, & He, 2008) (Matthaei, Piggot, & Townsend, 2010) and negatively affect non-target species such as macroinvertebrates that inhabit such ecosystem (Barmentlo, Schrama, van Bodegon, de Snoo, & Musters, 2019). However, no study has been undertaken to document the impact of rice farming practices on benthic macroinvertebrate assemblages in Ahero Irrigation Scheme and their response to varying levels of disturbance. Benthic environments are the most sensitive habitats of aquatic ecosystems to nutrient pollution, and changes in the structure of benthic communities are often extremely sensitive signs of enrichment (Cloem, 2001) (Gray, Wu, & Or, 2002). To adequately detect impairment in aquatic systems, it is necessary to monitor the biota (Downes, et al., 2002) as living communities respond to the entire range of biogeochemical factors in the environment (Karr & Chu, 2000). Mixtures of fertilizer and herbicide use are commonly found, yet these chemicals are rarely studied in combination. Insecticides may pose a particular risk to the aquatic environment as these compounds tend to co-occur, are persistent and highly water soluble, readily leaching from surrounding soils into the aquatic environment and, where present, are likely to cause adverse effects (Xu *et al.*, 2016; Health Canada, 2021a; 2021b; US EPA, 2023).
Studies have shown that pesticides can have a serious impact on biodiversity (Arimoro & Keke, 2016) due to their widespread application to reduce target animals, plants, and fungi in farmlands as well as non-target organisms (Ito, Shiraishi, Nakagawa, & Takamura, 2020). The most commonly perceived indirect pesticide effects in rice fields are reductions in species diversity, changes in community structure, and proliferation of selected species (Uddin, et al., 2016) (Ito, Shiraishi, Nakagawa, & Takamura, 2020). Earlier research established that pesticides indirectly affect the aquatic ecosystem by interrupting the aquatic food chain resulting in the loss/shift in abundance of natural species (Uddin, et al., 2016). However, at Ahero Irrigation Scheme, non-target effects of pesticides on aquatic organism in rice fields have received very little attention.

The recognition that invertebrates are essential components of freshwater, marine, and terrestrial environments has led to increased demand for conservation of their populations, and for the use of invertebrates as tools in ecological assessment and monitoring (New, 1988). Because the response of invertebrates to ecosystem changes varies among different taxa, estimates of diversity and abundance of those known to respond to certain factors are commonly used as indicators of habitat quality in comparing assemblages or sites (New, 1988) (Wallace & Webster, 1996). Invertebrates inhabiting the benthic environment have become particularly valuable in biomonitoring studies as most benthic invertebrates have short life cycles and respond rapidly to alterations in habitat (New, 1988). In addition, many benthic invertebrates are relatively sedentary, so are forced to either adapt to environmental stress or perish (Bilyard, 1987). Benthic infaunas, in particular, are superior to many other benthic groups for use in biomonitoring, as a result of their non-motile nature and importance to overall ecosystem structure and function (Bilyard, 1987). Members of the Ephemeroptera, Plecoptera, and Trichoptera (EPT) orders are known to be sensitive to water quality impairment, and as a result, the number of EPT taxa is commonly used as an indicator of low stress in bioassessment studies (Miltner & Rankin, 1998) (Maxted, et al., 1999) (Whiles, Brock, Franzen, & Dinsmore, 2000) (Sponseller, Benfield, & Valett, 2001) (Rungnapa, Baicha, & Tatporn, 2023).
Macroinvertebrate community responses to pollution and habitat degradation induced by agricultural land practices have received much attention and many studies have investigated faunal assemblage changes (Neumann & Dudgeon, 2002). However, specific data on the occurrence and population dynamics of aquatic organisms in agricultural areas are very limited. In Tanzania, (Alavaisha, Lyon, & Lindborg, 2019) showed that the sensitivity of macroinvertebrate to nutrient enrichment, particularly to ammonium-N and nitrate-N, is well known and reflected in water quality diversity since high nutrient concentrations decrease the level of oxygen in streams and decrease species richness. The study demonstrated only the relationships between ammonium-N and nitrate-N and macroinvertebrate community structure but failed to show any relation with phosphorous and other land use practices. Agrochemical application is one of the main management practices affecting diversity and abundance of aquatic organisms, not only by the direct

toxic effects, but also by changing the physico-chemical conditions of water (Rizo-Patrón et al. 2013) (Che Salmah, Siregar, Hassan, & Nasution, 2017) (Bao, et al., 2021). Furthermore, agricultural modernization over the last half-century such as agrochemical application and irrigation management has caused biodiversity losses in rice paddy ecosystems (Washitani, 2007) (Natuhara, 2013) (Yamamuro, et al., 2019). Very few studies have assessed impacts of agrochemicals on benthic macroinvertebrate communities in rice paddies and more so, none has been done in Ahero Irrigation Scheme. Therefore, this study looked at the influence of agrochemical application on benthic macroinvertebrate abundance and diversity.

3 MATERIALS AND METHODS

3.1 Location of Study Area

This study was conducted in Ahero Irrigation Scheme located in Muhoroni Sub-county, Kisumu County, Kenya (Fig 1). It lies in the Kano plains between Nandi Escarpment and Nyabondo Plateau at an altitude of 1,150 m above sea level between latitudes 34.90E and 34.97E, and longitudes 0.11S and 0.16S. According to the National Irrigation Board, Ahero Irrigation scheme was established in 1969 in the middle of the Kano Plains, on the eastern margin of the Winam Gulf of Lake Victoria, 25 km southeast of Kisumu City (Mwatet, 2016). The soil type found in this area is the black cotton soil (vertisol) and is rather fertile (Nyakach, 2019) but does not allow quick infiltration of surface water into the ground. This compounds the problem of drainage since surface drainage is already impeded by the gradient (Omuto, 2003).

The climate of the Kano plain is relatively dry and the average temperatures are high during the day (Nyakach, 2019) with annual mean temperatures varying between 17°C and 32°C. The area is relatively humid due to its proximity to Lake Victoria. It experiences three peaks of rains with an average annual rainfall of 1,000 – 1,800 mm and an average relative humidity of 65%. The first peak of rains occurs between March and July, with an average monthly rainfall of 150 – 260 mm. The other rainy season occurs in August. Short rains occur between September and October and have an average monthly rainfall of at least 125 mm. The dry period occurs between December and February. The irrigated area is supplied with water from River Nyando, where rice is planted in two seasons annually. The seasons often coincide with the local rainfall patterns; one crop is harvested in July and the other in January.

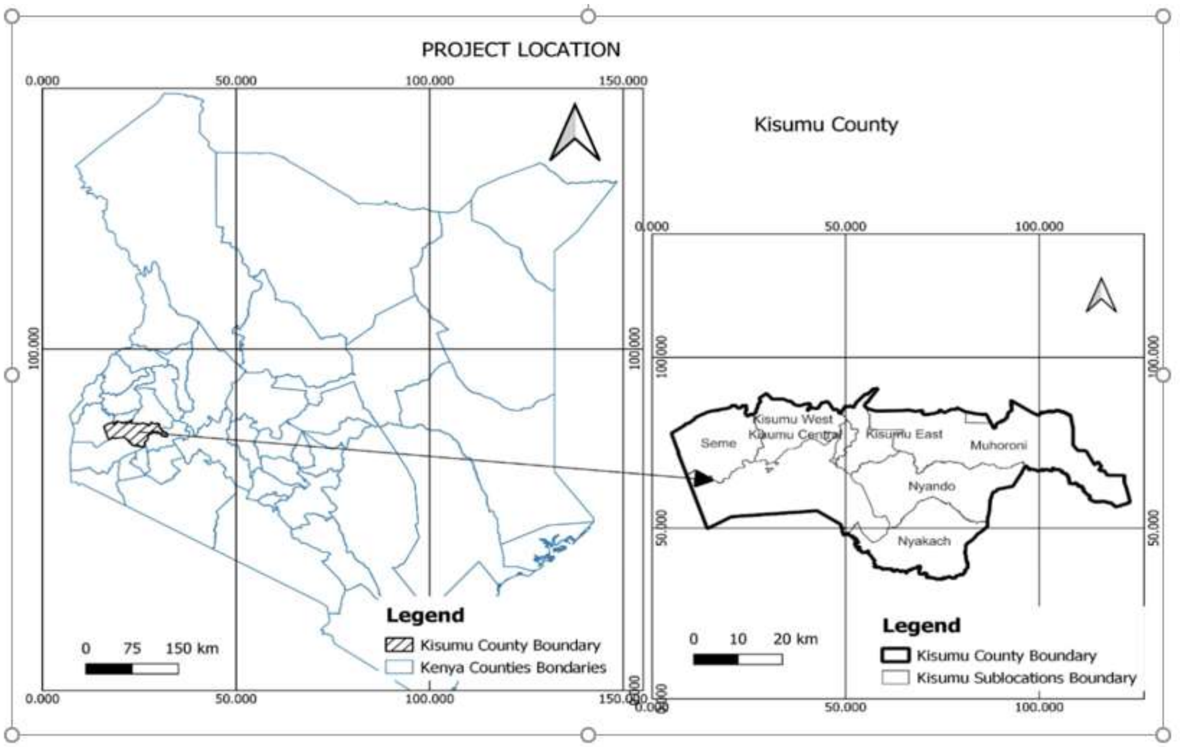

Fig 1: Map of Kenya showing Kisumu County

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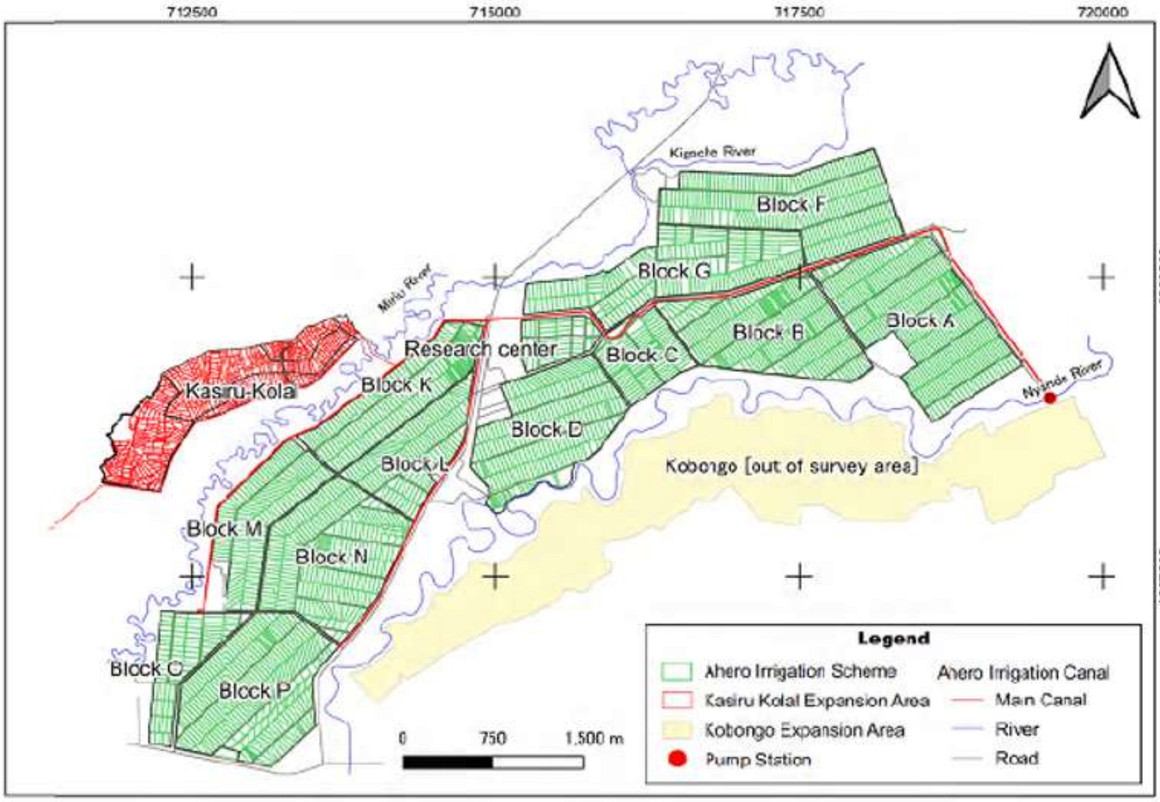

Fig 2: Map of Ahero Irrigation Scheme showing blocks from where the study plots were picked

3.2 Study Design

This study adopted descriptive longitudinal research design where purposive random sampling was used to systematically select 8 blocks from the 12 blocks that are in the scheme (Singh & Masuku, 2014) to help in determining homogeneity. A total of 18 sampling sites were randomly picked encompassing 16 cultivated rice fields, an inlet channel and an outlet channel (Appendix 1). The co-ordinates and altitude of each sampling site was determined using a handheld GPS (etrex 10 Garmin). Plots picked from blocks A, B, D & G had fields in which agrochemicals (fertilizers & pesticides) were applied while in blocks K, L, M & N no agrochemicals (fertilizers & pesticides) were applied in the fields from which samples were picked. The two channels, inlet and outlet were sampled to act as reference point and simulate natural environment (Appendix 1). From the time of ploughing, puddling upto nursery establishment the paddy fields were flooded moderately. Flooding was maximized (to a depth of about 30 cm) from the time of transplanting (4weeks after ploughing) to the second period of weeding (2 months after planting) before being gradually reduced (by about 10cm weekly) until total discontinuation at crop maturity (after 3 months). Ecological observations were made in plots with cultivated rice from where triplicate sampling was done, and also in the inlet and outlet channels. The dependent variable, benthic macro-invertebrate assemblages was checked against rice farming practices, which is the independent variable.

3.3 Study Sampling

Benthic macroinvertebrates were collected fortnightly for six months. This period entailed part of the dry season (October, November and December) and part of the wet season (March, April and May) covering two rice crop development seasons and also corresponded to the stages of land preparation, germination, growth, reproduction and maturity.

3.3.1 Sampling of benthic macroinvertebrate assemblages

During sampling, triplicate samples of benthic macro invertebrates were taken from each site, during the rice developing cycle in the randomly selected rice paddies. For each station, sampling was repeated at three points to ensure objectivity. Sampling was done using Ekman grab to collect macroinvertebrate samples living submerged in the water and those living on the surface of sediments and water surface. Water and sediment samples were poured into the layered strainer with different sizes in order to sift the sediments from the samples and transferred into collecting bottles filled with 70% ethanol using a brush before being transported to the laboratory for storage awaiting identification. Specimens were sorted and identified to the lowest possible and practicable taxonomic category and counted using a dissecting microscope in the laboratory. For identification, the Merritt & Cummins (1996), Bond-

Buckup & Buckup (1999) and Fernandes & Domingues (2001) identification keys were used. Ecological indices were selected to describe the diversity of each station.

3.4 Statistical Analysis

Data entry was done using Microsoft Excel spreadsheet while statistical data analysis was done using IBM SPSS Statistic 21. One-way ANOVA at $\alpha = 0.05$ was used to test for and compare significance differences in the variables between stations. Measure of association was determined using Eta squared (Eta^2) to show the effect size where values between 0 to 13% was considered small (weak), 14 to 26% was medium (moderate) and above 26% was considered to be strong (large) (Cohen J. , 1988) (Cohen, Cohen, West, & Aiken, 2003). For differences that were found significant at p = 0.05, a post hoc separation of means was done by Duncan's Multiple Range Test (DMRT). Biodiversity indices such as species diversity {Shannon-Wiener, (H′) index}, species evenness {Pielou's Evenness, (J)} and species dominance {Simpson dominance, (D)} were calculated for each sample corresponding to each station and locality using Excel spreadsheet Software. The differences in faunal occurrence (order and families) with regards to information on abundance and diversity of macroinvertebrates in the paddies were obtained. The results were discussed in relation to the objective and presented in textual form, tables and graphs.

4. RESULTS

Macroinvertebrate Abundance

A total of 22414 individuals distributed among 29 macroinvertebrate taxa were collected from the sampled rice fields of which 10919 and 11495 individuals were collected during dry and wet seasons respectively (Appendix 2) from 3 phyla Arthropoda (insecta and arachnida), Annelida (oligochaeta and clitellate) and Mollusca (gastropoda and Bivalvia). From the total macroinvertebrates collected, 18, 776 were from class insecta, belonging to 5 orders and 21 families representing 84% of the total individuals collected. In this study it was found that the more abundant insect orders were Hemiptera 30% (5,632), Diptera 28% (5,199), Coleoptera 27% (5,158), Ephemeroptera 9% (1,630) and Odonata 6% (1,157). The most abundant individual taxa (table 2) in this study during the two seasons were Chironomidae (2186) 10.4%, Gyrinidae (1714) 7.9% and Belostomatidae (1643) with 7.2% while the least abundant families were gomphidae(122) and unionidae(89)(Appendix 2). The total mean abundance for benthic macroinvertebrates varied significantly (One-Way ANOVA at $\alpha = 0.05$, $F_{(5,114)} = 100.440$, $p = 0.000$) during various operations in the rice fields. Duncan Multiple Range Test (DMRT) further established that the means during land preparation and transplanting were significantly different from the other operations. However, there were no significant statistical differences in mean abundance value during first weeding, fertilizer application, second weeding and onset of maturity. The highest value (245.60±40.674) was recorded immediately after fertilizer application at flowering stage and the lowest mean value (81.3±15.26) was recorded during land preparation, nursery establishment and seedbed preparation(table 1).

Table 1: Mean values for macroinbertebrates abundance during different operations

Operation	Macroinvertebrate abundance (Mean ± SD)
Land preparation, nursery establishment and seedbed preparation	81.3 ± 15.26^{A}
Transplanting	94.35 ± 18.12^{A}
First weeding and pesticide application	228.05 ± 36.14^{B}
Fertilizer application at flowering	245.6 ± 40.67^{B}
Second weeding and pesticide application at ear formation	237.6 ± 42.72^{B}
Onset of maturity and beginning of cut	233.65 ± 41.58^{B}
MEAN	186.76±78.14

*Values represent means ± Sd of triplicate analysis. **Means with different superscripts in the same column are significantly different at $\alpha = 0.05$ (Means separated by DMRT)

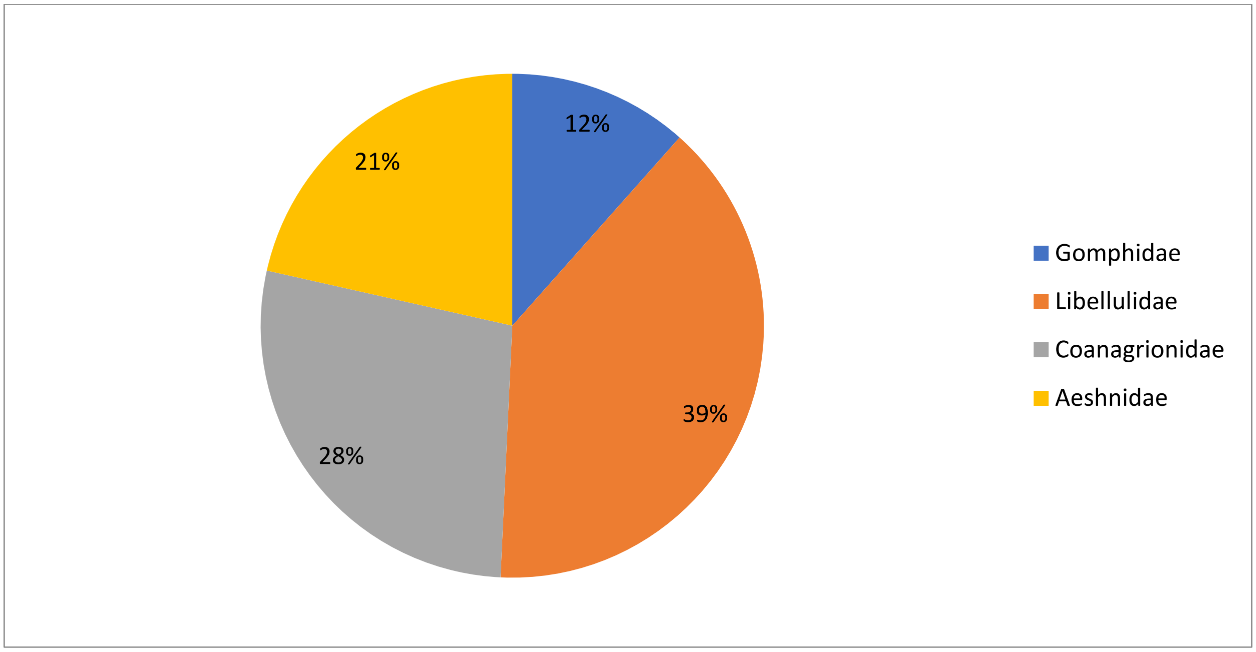

Figure 4: Pie chart showing percentage composition and abundance of family members in the order Odonata

The order Odonata was represented by the families Gomphidae, Libellulidae, Coenagrionidae and Aeshnidae with libellulidae being the most abundant (3.69±2.300) while gomphidae was the least abundant (1.02±0.898) (Fig 4.4). There was significant difference in mean number for all the family members of the order Odonata (One-Way ANOVA at α =0.05, p = 0.000) during different operations (Appendix 7). For the gomphidae family Duncan Multiple Range Test (DMRT) established that the mean during land preparation and transplanting significantly differed from each other and other operations where there was no significant statistical difference amongst first weeding, fertilizer application, second weeding and onset of maturity. Libellulidae family varied significantly (One-Way ANOVA at α =0.05, $F_{(5,114)}$ = 36.334, p = 0.000). Duncan Multiple Range Test (DMRT) further established that the means during first weeding and fertilizer application significantly differed statistically from each other and the other operations (Table 4.8). However, there was no statistical significant difference between land preparation and second weeding at ear formation and onset of maturity (Table 4.8). Coanagrionidae and aeshnidae showed a similar pattern with each other as land preparation and transplanting were significantly different from the other operations but not from each other (Table 4.8).

Table 4: Mean abundance of families in the orders Odonata & Ephemeroptera

	Mean values of families in orders Odonata & Ephemeroptera (Mean ± SD)					
Operation	Gomphidae	Libellulidae	Coanagrionidae	Aeshnidae	Baetidae	Caenidae
Ploughing	0.30±0.470^{A}	1.10±.641^{A}	0.90±0.718^{A}	1.30±1.081^{A}	0.85±0.813^{A}	0.75±0.639^{A}
Transplanting	0.60±0.503^{AB}	1.40±.940^{A}	1.30±0.733^{A}	1.05±0.686^{A}	1.15±0.745^{A}	1.00±0.562^{AB}
First weeding	1.20±0.894^{C}	4.05±1.276^{B}	2.80±1.361^{B}	2.20±1.105^{B}	17.00±3.212^{B}	16.80±3.088^{C}
Fertilizer application	1.10±0.718B^{C}	4.80±1.361^{BC}	3.70±1.342^{C}	2.50±1.100^{B}	18.05±4.045^{B}	16.95±4.861^{C}
Second weeding	1.30±0.979^{C}	5.10±1.518^{C}	4.10±1.334^{C}	2.90±1.294^{B}	2.10±0.968^{A}	2.45±1.146^{B}
Onset of maturity	1.60±1.046^{C}	5.70±2.386^{C}	4.10±1.294^{C}	2.75±1.209^{B}	2.25±1.020^{A}	1.10±0.718^{AB}
MEAN	1.02±0.898	3.69±2.30	2.82±1.73	2.12±1.29	6.90±7.85	6.51±7.76

*Values represent means ± Sd of triplicate analysis. **Means with different superscripts in the same column are significantly different at α= 0.05(Means separated by DMRT)

The order Ephemeroptera was represented by Baetidae (6.90±7.876) and Caenidae (6.51±7.58) families. They showed that there were significant statistical differences in mean (One-Way ANOVA at α =0.05 and p-value > 0.05) abundance during different operations (Appendix 7). Baetidae family varied significantly (One-Way ANOVA at α =0.05, $F_{(5,114)}$ = 273.680, p = 0.000). Duncan Multiple Range Test (DMRT) established that the mean distribution during first weeding and fertilizer application differed significantly from land preparation, transplanting, second weeding and onset of maturity. Caenidae family varied significantly (One-Way ANOVA at α =0.05, $F_{(5,114)}$ = 217.814, p = 0.000). Duncan Multiple Range Test (DMRT) further established that transplanting and onset of maturity differed significantly from the other operations but did not differ from each other (Table 4.8). In terms of mean abundance, orders Odonata and Ephemeroptera showed low abundance of the total insects collected and there was complete absence of Trichoptera and Plecoptera in the current in Ahero rice fields.

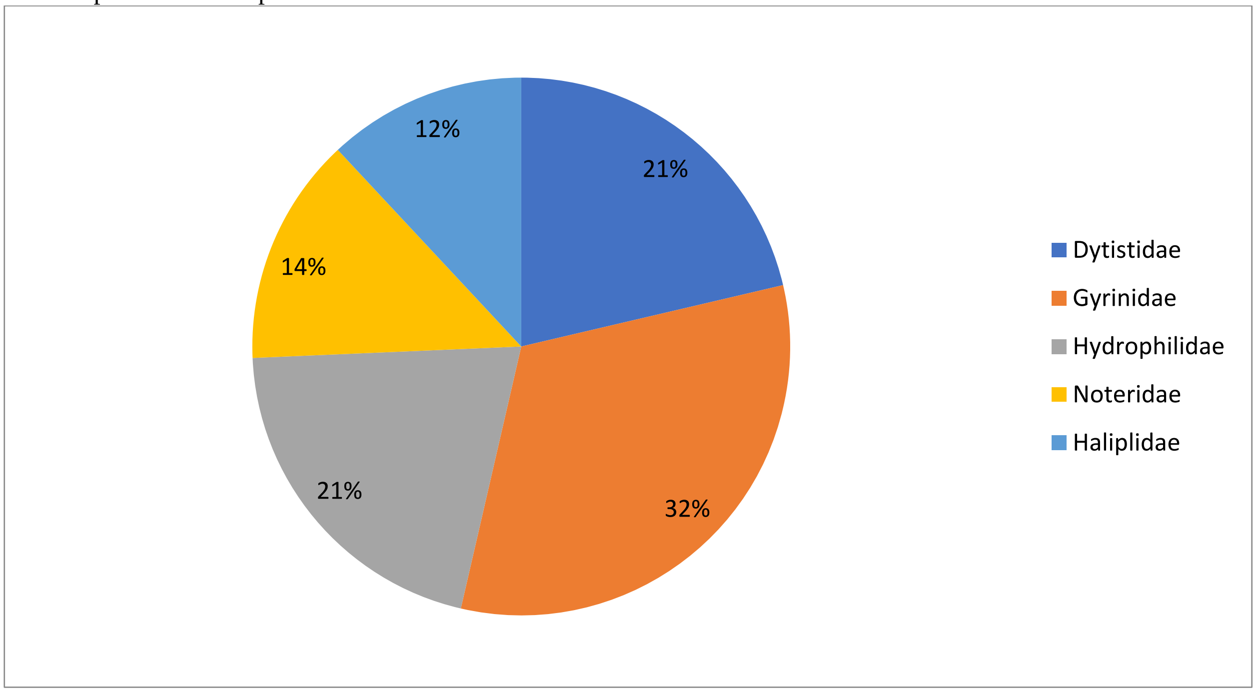

Figure 4.2: Pie chart showing percentage composition and abundance of family members of order Coleoptera

Order Coleoptera was represented by families Dytistidae, Gyrinidae Hydrophilidae, Noteridae and Haliplidae. Family Gyrinidae (1,714 individuals) was most abundant with a total means of 14.18±5.843 (32%) and the least abundant being Haliplidae (592 individuals) with a total mean of 4.93±2.758 (12%) (Fig4.2). The coleopterans showed significant statistical differences in mean (One-Way ANOVA at α =0.05 and p-value > 0.05) distribution during different operations (Appendix 7). Gyrinidae family varied significantly (One-Way ANOVA at α =0.05, $F_{(5,114)}$ = 62.756, p = 0.000). Duncan Multiple Range Test (DMRT) established that the mean distribution during land preparation and transplanting had a significant statistical difference from all the operations and not from each other (Table 4.10). First weeding, fertilizer application and second weeding did not have significant statistical difference from each other. For the family haliplidae transplanting significantly differed statistically from all the operations. However, first weeding, fertilizer application, second weeding and onset of maturity did not show any statistical significant difference (Table 4.10).

Table 4.10: Abundance and mean difference of families in the orders Coleoptera

Operation	) Dytistidae	Gyrinidae	Hydrophilidae	Noteridae	Haliplidae
Ploughing	$3.95±1.761^{A}$	$6.75±1.682^{A}$	$3.65±1.268^{A}$	$2.10±0.912^{A}$	$1.50±0.827^{A}$
Transplanting	$5.75±1.860^{B}$	$7.55±1.877^{A}$	$4.25±1.251^{A}$	$3.10±1.586^{A}$	$2.00±1.026^{AB}$
First weeding	$9.65±2.159^{C}$	$17.00±2.991^{B}$	$8.90±1.714^{B}$	$6.60±1.429^{B}$	$5.95±1.669^{B}$

Fertilizer application	10.55±1.605^{C}	17.95±3.069^{B}	10.05±1.959^{B}	7.55±2.038^{B}	6.90±1.997^{B}
Second weeding	13.55±3.236^{D}	17.80±3.503^{B}	12.95±3.034^{C}	7.05±1.395^{B}	6.50±1.539^{B}
Onset of maturity	13.35±3.200^{D}	18.00±4.472^{B}	13.00±2.938^{C}	6.85±2.183^{B}	6.75±2.023^{B}
MEAN	9.47±4.30	14.18±5.843	8.80±4.301	5.54±2.669	4.93±2.758

*Values represent means ± Sd of triplicate analysis. **Means with different superscripts in the same column are significantly different at α= 0.05(Means separated by DMRT)

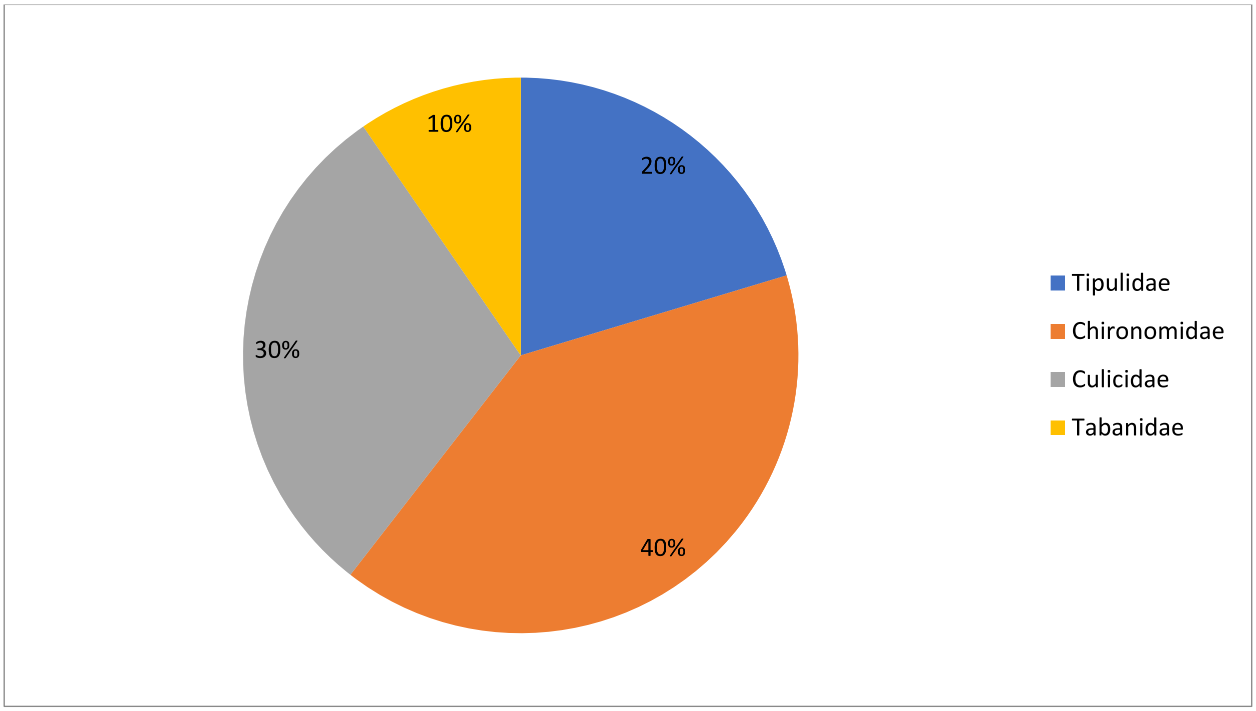

Figure 4.3: Pie chart showing percentage composition and abundance of family members in the order Diptera

The order Diptera was represented by tipulidae, chironomidae, culicidae and tabanidae (Fig4.3). All the dipterans showed a significant statistical difference in mean (One-Way ANOVA at α =0.05 and p-value > 0.05) distribution during different operations (Table 4.11). Chironomidae family varied significantly (One-Way ANOVA at α =0.05, $F_{(5,114)}$ = 64.290, p = 0.000). Duncan Multiple Range Test (DMRT) established that the mean distribution during land preparation and transplating significantly differed from all the other operations but not from each other (Table 4.12). There was significant difference in mean number for family tabanidae (One-Way ANOVA at α =0.05, $F_{(5,114)}$ = 55.018, p = 0.000). A post hoc test by Duncan Multiple Range Test (DMRT) further established that the mean during land preparation and transplanting showed significant difference from the periods when chemicals were applied and the onset of maturity (Table 4.12). The families Tipulidae and culicidae showed similar trend (One-Way ANOVA at α =0.05 and p = 0.000) (Table 4.11). When post hoc test was done the DMRT revealed that during land preparation and transplanting there was significant statistical difference from the time of chemical application and onset of maturity but not from each other (Table 4.12).

Table 4.12: Abundance and mean difference of families in the orders Diptera & Hemiptera

	Temporal distribution of families in orders Diptera & Hemiptera (Mean ± SD)					
	Tipulidae	Chironomidae	Culicidae	Tabanidae	Belostomatidae	Corixidae

Operation						
Ploughing	2.65±1.182^{A}	9.10±3.370^{A}	6.85±2.889^{A}	1.00±.725^{A}	6.90±1.651^{A}	4.30±1.455^{A}
Transplanting	3.75±1.482^{A}	11.10±3.354^{A}	7.70±2.975^{A}	1.25±.716^{A}	7.65±2.084^{A}	4.95±1.932^{A}
First weeding	9.60±1.698^{B}	22.65±4.404^{B}	17.20±3.222^{B}	4.95±1.276^{C}	14.75±2.511^{B}	10.60±1.536^{B}
Fertilizer application	10.80±2.167^{B}	23.10±3.669^{B}	18.20±3.518^{B}	5.35±1.387^{C}	14.90±3.210^{B}	10.70±2.105^{B}
Second weeding	9.85±2.300^{B}	21.70±3.294^{B}	16.30±3.028^{B}	5.25±1.333C	20.00±3.387^{C}	13.40±2.280^{C}
Onset of maturity	9.75±2.573^{B}	21.75±3.007^{B}	16.20±3.286^{B}	4.10±1.553^{B}	19.20±3.443^{C}	14.30±3.310^{C}
MEAN	7.73±3.781	18.23±6.78	13.74±5.58	3.65±2.19	13.90±5.79	9.71±4.41

*Values represent means ± Sd of triplicate analysis. **Means with different superscripts in the same column are significantly different at α= 0.05(Means separated by DMRT)

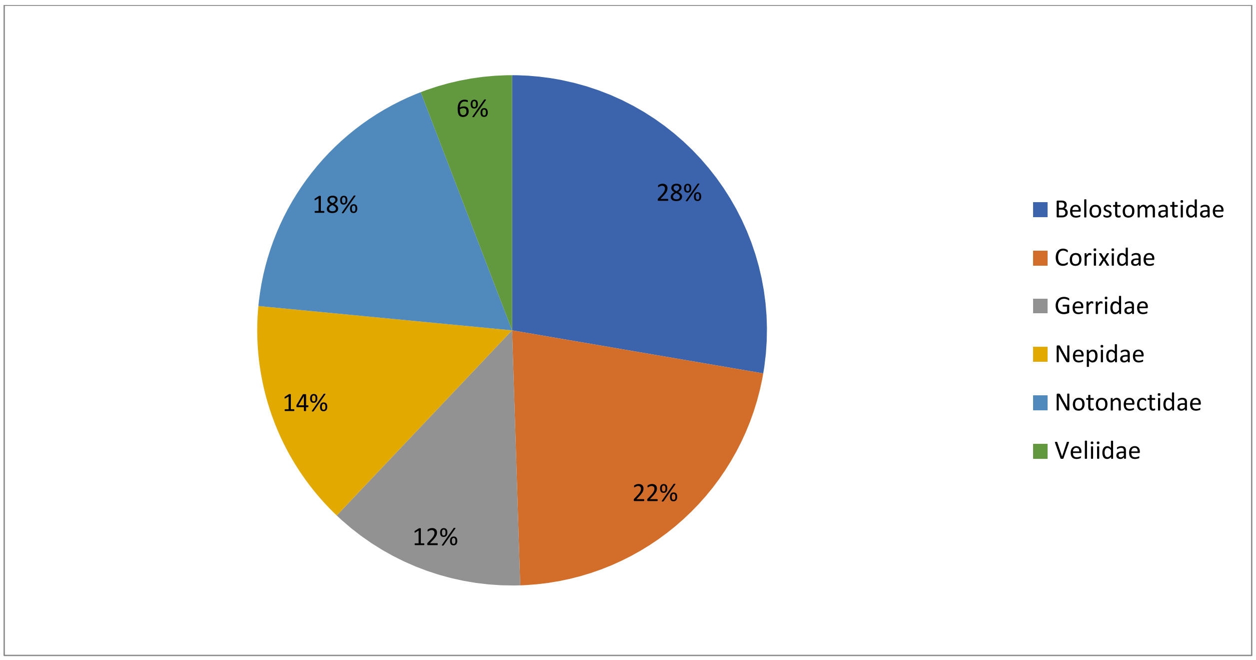

Figure 4.1: Pie chart showing percentage composition and abundance of family members of order Hemiptera

The order Hemiptera being the most abundant in this study was represented by six families (Belostomatidae, Corixidae, Gerridae, Nepidae, Notonectidae and Veliidae), with the most abundant family being Belostomatidae (1,643 individuals) with a total means of 13.90±5.794 (28%) and the least abundant being Veliidae (166 individuals) with a total mean of 2.43±1.268 (6%) of the total hemipterans (Fig 4.1). They showed a significant statistical difference in mean (One-Way ANOVA at α =0.05 and p-value > 0.05) distribution during different operations (Appendix 7). Veliidae family varied significantly (One-Way ANOVA at α =0.05, $F_{(5,114)}$ = 11.519, p = 0.000). Duncan Multiple Range Test (DMRT) further established that the mean distribution during land preparation and transplating significantly differed from each other, the mean value during chemical applications also differed from each other and from onset of maturity (Table 4.14). The families corixidae, geridae, nepidae and notonectidae showed similar trend (One-Way ANOVA at α =0.05 and p = 0.000). When post hoc test was done the DMRT revealed that during land preparation and transplanting there was significant statistical difference from the time of chemical application and onset of maturity but not from each other (Table 4.14).

Table 4.14: Temporal distribution and mean difference of families in the orders Hemiptera & Acari

Temporal distribution of families in orders Hemiptera & Acari (Mean ± SD)

International Journal of Academic Management Science Research (IJAMSR)
ISSN: 2643-900X
Vol. 9 Issue 4 April - 2025, Pages: 55-79

Operation	Gerridae	Nepidae	Notonectidae	Veliidae	Acaricidae	Hydrachnidae
Ploughing	2.80±1.056^{A}	3.20±1.005^{A}	3.70±1.218^{A}	1.45±0.945^{A}	3.55±1.191^{A}	4.20±1.473^{A}
Transplanting	2.95±1.050^{A}	3.55±1.356^{A}	4.15±1.496^{A}	1.65±0.988^{AB}	3.50±1.235^{A}	4.60±1.188^{A}
First weeding	6.35±1.599^{B}	7.20±1.508^{B}	8.55±1.572^{B}	2.30±1.031^{BC}	10.45±1.849^{C}	9.40±2.010^{B}
Fertilizer application	6.60±1.698^{B}	7.75±1.713^{B}	9.35±2.254^{B}	2.55±0.945^{C}	10.90±2.404^{C}	9.80±2.587^{B}
Second weeding	8.60±1.818^{C}	9.75±1.713^{C}	12.20±1.881^{C}	3.40±1.188^{D}	7.55±1.849^{B}	5.35±1.599^{A}
Onset of maturity	8.60±1.903^{C}	10.10±2.532^{C}	11.80±3.318^{C}	3.25±1.209^{D}	7.35±2.560^{B}	4.60±1.465^{A}
MEAN	5.98±2.82	6.93±3.19	8.29± 3.92	2.43±1.27	7.22±3.49	6.33±2.93

*Values represent means ± Sd of triplicate analysis. **Means with different superscripts in the same column are significantly different at α= 0.05(Means separated by DMRT)

The Acari consisting of two families; acariciidae and hydrachnidae (Appendix 4 & 6) showed significant statistical differences in mean (One-Way ANOVA at α =0.05 and p-value > 0.05) distribution during different operations (Appendix 7). Post hoc test by Duncan Multiple Range Test (DMRT) established that the mean during first weeding and fertilizer application significantly differed from all other operations but not from each other (Table 4.16). For acaricidae, the mean for second weeding also showed significant statistical difference from the other operations. Macroinvertebrate families representing the molascans showed significant difference in mean (One-Way ANOVA at α =0.05 and p < 0.000) during all operations (Appendix 7). Duncan Multiple Range Test (DMRT) established that during second weeding and onset of maturity there was a significant statistical difference from all the other operations for all the families (Table 4.16).

Table 4.16 showing temporal distribution and mean difference of families in the orders Naidomorpha, Huridinea, Prosobrancha & Sphaeridae

	Temporal distribution of families in orders Naidomorpha, Huridinea, Prosobrancha & Sphaeridae (Mean ± SD)					
Operation	Tubificidae	Hurididae	Hydrobidae	Physidae	Thiaridae	Unionidae
Ploughing	2.00±0.973^{A}	0.70±0.571^{A}	3.25±1.682^{A}	1.50±0.827^{A}	1.00±0.649^{A}	0.45±0.510^{A}
Transplanting	2.85±1.387^{A}	1.00±0.649^{A}	3.65±1.461^{A}	1.60±0.821^{A}	0.95±0.605^{A}	0.60±0.598^{A}
First weeding	4.45±1.731^{B}	2.05±0.826^{B}	4.10±1.744^{A}	1.75±0.716^{A}	1.15±0.671^{A}	0.55±0.686^{A}
Fertilizer application	4.95±1.731^{B}	2.10±0.912^{B}	4.25±2.221^{A}	1.70±0.733^{A}	1.00±0.562^{A}	0.75±0.716^{AB}
Second weeding	4.75±1.773^{B}	2.20±1.005^{B}	11.60±2.437^{B}	4.05±1.276^{B}	2.65±0.933^{B}	0.90±0.718^{AB}
Onset of maturity	3.95±1.191^{B}	2.15±1.040^{B}	11.15±2.870^{B}	4.45±1.317^{B}	2.75±1.293^{B}	1.10±0.641^{B}
MEAN	3.83±1.81	1.70±1.034	6.33±4.16	2.51±1.57	1.58±1.14	0.73±0.67

*Values represent means ± Sd of triplicate analysis. **Means with different superscripts in the same column are significantly different at α= 0.05(Means separated by DMRT)

In the different times corresponding with different operations, there were significant statistical difference (One-Way ANOVA at $\alpha = 0.05$, $F_{(5,114)} = 2.782$ and $p = 0.021$) in the means for unionidae during all operations (Appendix 7). Duncan Multiple Range Test (DMRT) established that mean during land preparation, transplanting and first weeding significantly differed from all other operations but not each other (Table 4.16). Similarly, there were significant statistical differences in mean values during fertilizer application and second weeding from all other operations but not from each other. Onset of maturity also showed significant statistical difference from all other operations.

Benthic Macroinvertebrate Diversity Indices

The biodiversity indices are presented in Tables 4.5 and 4.6 for the different sites located on the eastern and western side of the Research Station respectively. In sites located on the eastern (A, B, D & G), Taxa richness and Pielou's evenness portrayed similar trends across sites, each revealing higher Shannon Diversity values in site A (3.108) and B (3.132), and lower in D (3.093) and G (3.075). There was no significant difference in Pielou's evenness (J) index values in site A (0.923), B (0.930), D (0.919) and G (0.0.913) as well as taxon richness (S) as it varied slightly site A (27), B (29), D (29) and G (28). For sites located on the western side, the Shannon Diversity varied at M (3.156), N (3.134), K (3.080) and L (3.059). Pielou's evenness (J) for these plots was K (0.926), L (0.916), M (0.937), and N (0.926) with varied taxon richness {K (29), L (28), M (29) and N (29}.

Table 4.5 (a): The biodiversity indices for dry season during land preparation

Biodiversity	Sampling sites									
index	Inlet	Plot A	Plot B	Plot D	Plot G	Plot K	Plot L	Plot M	Plot N	Outlet
Shannon-wiener	3.020	3.108	3.132	3.093	3.075	3.080	3.059	3.156	3.134	2.881
Pielou's	0.897	0.923	0.930	0.919	0.913	0.915	0.908	0.937	0.931	0.856
Abundance	121	160	185	178	189	195	181	183	185	70
Taxon richness	25	27	29	29	28	29	28	29	29	22

Note: Plots A, B, D, and G-Fertilizer and Herbicide were used

Plots K, L, M and N- No Fertilizer and Herbicide were used

5. Discussion

A total of 22414 individuals distributed among 29 macroinvertebrate families were collected from the sampled rice fields of which 10919 and 11495 individuals were collected during short and long rain growing cycles respectively (appendix 1). In a study carried out in the Itajaí Valley, Santa Catarina State, South Brazil (Molozzi, Hepp, & Dias, 2007) a total of 21,831 organisms, distributed in 28 families were collected with Chironomidae (Diptera) (73.92%), Baetidae (Ephemeroptera) 5.95% and Elmidae (Coleoptera) 2.50% being the most representative taxa. However, (Wakhid, Rauf, Krisanti, Sumertajaya, & Maryana, 2020) in their study of species richness and diversity of aquatic insects in Indonesia recorded a total of 3,306 individuals belonging to 20 families and 7 orders. These finding are testament to observation that rice fields are an ecosystem with high biological diversity compared to other agricultural areas (Stenert, Maltchik, & Rocha, 2012). In the inlet drainage, high abundance was recorded since it contained clean water compared to cultivated plots which were disturbed due to the various rice farming practices. During the two rice crop cycles plots K, L and M recorded higher values in terms of abundance. However highest abundances were captured during the wet season due to the dilution effect of the rains and availability of macroinvertebrate food. Other studies agreed that 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 (Bambaradeniya C. N., 2000) (Leitao, Pinto, Pereira, & Brito, 2007) (Asghar, 2010). The Outlet drainage contained heavy pollution load which negatively affected macroinvertebrate abundance. This especially contributed to the absence of pollution sensitive members of the Ephemeroptera, Plecoptera and Trichoptera families (Rosenberg, Resh, & King, 2008). This also explains the low abundance of Ephemeroptera, Odonata, Prosobrancha, Acari and Huridinae in Ahero Irrigation Scheme rice fields.

Total mean values of benthic macroinvertebrates abundance during various operations in the rice fields varied significantly (One-Way ANOVA at $\alpha = 0.05$, $F_{(5,114)} = 100.440$, $p = 0.000$). Duncan Multiple Range Test (DMRT) further establishes that the means during land preparation and transplanting were significantly different from the other operations. However, there were no significant statistical differences in mean abundance value during first weeding, fertilizer application, second weeding and onset of maturity. The highest value (245.60±40.674) was recorded immediately after fertilizer application at flowering stage and the lowest value (81.3±15.26) was recorded during land preparation, nursery establishment and seedbed preparation (Table 4.4). During the early paddy field growth stage, the abundance was slightly lower. The lower macroinvertebrate abundance during land preparation could have been due to few available water patches which serve as the only habitable areas for benthic organisms. Earlier on (Aspbury & Juliano, 1998) observed that limited habitable area, competition and other abundance-dependent factors led to a reduced numerical abundance in fallow season. The lower macroinvertebrate might also be associated with low benthic productivity due to unprocessed organic matter (Mitsch & Gosselink, 2007). After the application of insecticides during the end of transplant-young phase and beginning of tiller phase, the abundance of aquatic organisms declined again. Further, during the mature phase when there was complete shading of the water surface prior to grain harvesting, there was reduced abundance of aquatic organisms due to very low water levels in rice fields.

In this study there was a decline in abundance particularly for members of ephemeroptera (baetidae and caenidae) and diptera (particularly chironomidae) at the onset of maturity and beginning of cut thus a net negative effect on the general macroinvertebrate abundance. The abundance also declined in the late rice growth stage might have been due to the end of field flooding (Nachuha, 2009), (Ma, Cai, Li, & Chen, 2010) and deteriorating water quality (Sinha, Hazra, & Khan, 2011). Very low water levels 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 and Chironomidae (Che Salmah, Siregar, Hassan, & Nasution, 2017).

In this study we found that the more abundant insect orders were Hemiptera 30% (5,632), Diptera 28% (5,199), Coleoptera 27% (5,158), Ephemeroptera 9% (1,630) and Odonata 6% (1,157) (appendix 1). In their study (Munira & Rasel, 2012) found that hemipterans were diverse, abundant and important pests in the paddy ecosystem due to their piercing and sucking mouthparts that feed on the crop causing significant losses in rice yields. This explains why the hemipterans were the most abundant insect order in rice paddies as was the case in this study. In a similar study in Kilombero, Tanzania (Alavaisha, Lyon, & Lindborg, 2019), macroinvertebrates belonging to ten orders and 41 families were identified where Hemiptera, Odonata and Ephemeroptera were the most dominant contributing 24%, 18% and 13%, respectively, of the total macroinvertebrate taxa abundance. Further in Indonesia (Wakhid, Rauf, Krisanti, Sumertajaya, & Maryana, 2020) found that among the aquatic insect fauna inhabiting rice fields, the order Hemiptera was the most abundant comprising 28.89% of the total insects collected, followed by Diptera (24.80%), Coleoptera (24.41%), and Odonata (21.42%). The predatory nature of Hemipterans might have also made them the most abundant, thus giving them an edge over other families. Studies done by (Asghar, 2010); (Hayasaka, Korenaga, Sanchez, & Goka, 2012) have shown that hemipterans, coleopterans and odonates are either prey or predators, and that they live on insects such as baetids, corixids, notonectids, and hyrophilids (Mogi, 2007); (Varela & Gaput, 2013). Thus some of them occurred in high abundances in various rice cultivation phases (Mogi, 2007) (Al - Shami, Che-Salmah, Ahmad, & Aziza, 2010) and (Lupi D. R. A., 2013).

The abundance of odonata compared to hemiptera was low. This was due to the occurrence of a thin water layer and the lack of puddles during drying periods which critically reduced the suitability of rice fields for aquatic organisms, forcing them to emigrate elsewhere (Fasola & Ruiz, 2015). However, in a study of aquatic organisms in rice crops to evaluate the role of Uruguayan rice agroecosystems in biodiversity conservation of aquatic organisms (Bao, et al., 2021) found that the more abundant insect orders were Diptera (59.9%), Hemiptera (16.3%) and Ephemeroptera (14.0%). The low abundance of Ephemeroptera and the absence of Trichoptera and Plecoptera as recorded in the rice fields was as a result of their being sensitive to organic pollution as they live mainly in clean and well-oxygenated waters (Rosenberg et al. 2008). This was contrary to (Che Salmah, Siregar, Hassan, & Nasution, 2017) who reported a high proportion (2797%) of mayflies (Ephemeroptera: Baetidae) in rice fields in North Sumatera, and (Thongphak & Iwai, 2016) in Thailand. In their study (Molozzi, Hepp, & Dias, 2007) further found that diptera was common in all the crop areas and growing stages, being that in all the sites, these organisms were significantly more abundant (7,346 ind/m2; F5,12 = 4.22; p = 0.01).

From the results obtained chironomidae was common in all the crop areas and growing stages (Molozzi, Hepp, & Dias, 2007). Earlier study established that the great abundance of chironomidae family was due to the fact that some genera were capable to live in condition of total depletion of oxygen for some hours (Kleine & Trivinho-Strixino, 2005). Chironomidae also presents great environmental plasticity, living where other more sensible organisms, as the Ephemeroptera, Plecoptera and Trichoptera (EPT) are absent. This explains the low density of Ephemeroptera and absence Plecoptera and Trichoptera in this study during all the cycle of rice crop. Also (Suhling, et al., 2000) commented that the low density of organisms of the EPT is due to the insecticides application and the high concentrations of nitrogen and phosphorus. Still (Mesleard, Garnero, Beck, & Rosecchi, 2005) cited that in systems of organic production of rice, Ephemeroptera and Coleoptera were the most abundant organisms, while chironomidae predominated in systems of conventional management (application of chemical products). Furthermore, chironomidae have also been utilized as indicators of nutrient enrichment in water and sediment (Al-Shami, Che-Salmah, Ahmad, Hamid, & Nor, 2011) in Malaysia.

Negative effects of pesticides on aquatic organisms in rice fields have been previously documented by various authors (Schoenly, Justo, Barrion, & Bottrel, 1998); (Suhling, et al., 2000) & (Wilson, Watts, & Stevens, 2008). The application of chemicals such as fertilizers, herbicides and insecticides often leads to nutrient enrichment of surface waters (Dudgeon, 2000); (Jergentz, Pessacq, Mugni, Bonetto, & Shulz, 2005) & (Baumart & Santos, 2011)consequently, the abundance of aquatic organisms were much reduced at these times as was the case in this study. In growth and reproduction stages of rice, an intense application of chemicals occur in the fields (Molozzi et al., 2006).The presence of pesticide in the water surface can result in a reduction of macroinvertebrates diversity due to the elimination of the less tolerant organisms, allowing the generalist organisms, an expansion and predominance in the environment (Kellog, 1994); Carballo, 2003; (Pastor, Sanpera, Gonzales-Solis, Ruiz, & Albaiges, 2004) & (Douglas & O'Connor, 2005). In the present study, similar condition was recorded since diversity in these periods was low due to the pesticide application in the rice fields. From this study it was evident that the stage of growth and reproduction presented highest density and average richness while during maturation and cut, highest average values were found for the diversity and evenness. Similar result was found in a study carried out in the Itajaí Valley, Santa Catarina State, South Brazil (Molozzi, Hepp, & Dias, 2007) where highest density and average richness (3,435 ind/m2 and 27 taxa, respectively) were recorded during growth and reproduction and highest average values were found for the diversity and evenness (2.146 and 0.533, respectively) during maturation and cut. The most abundant individual taxa in this study were Chironomidae (Diptera) 10.4%, Gyrinidae (Coleoptera) 7.9% and Belostomatidae (Hemiptera) with 7.2% (Appendix 1, 2 &3). This was in agreement with the findings of (Wang, Xu, Yang, Shen, & Yu, 2007); (Zhao, Wang, & Close, 2012); (Zhang, et al., 2014); (Rosser & Pearson, 2018); and (Shabani, Liu, Yu, Muhigwa, & Geng, 2019) who reported that insects represented the most diverse group and Chironomidae were the most abundant family. Order Prosobrancha was represenred by Hydrobiidae (6.33±4.155), Physidae(2.51±1.572) and Thiaridae(1.58±1.135). There was a remarkable increase in abundance for hyrdobiidae after application of agrochemicals (Appendix 2 & 3). However, there was a reduction in abundance for some families after application of agrochemicals after the beginning of tiller phase (Apendix 2& 3). This agreed with other studies that had documented negative effects of pesticides on aquatic organisms in rice fields (Schoenly, Justo, Barrion, & Bottrel, 1998); (Suhling, et al., 2000) and (Wilson, Watts, & Stevens, 2008).

Macroinvertebrate richness in Ahero Rice Fields (29 taxa) was low compared with the richness observed in rice fields in Thailand (183 species) by Heckman (1979), Sri Lanka (154 species) by (Bambaradeniya, et al., 2004) but it was higher than the number of species found in Malaysia (19 species) by Lim (1980) and India (26 species) by (Roger, Grant, Reddy, & Watanabe, 1987). This might have been due to unchecked pollution in the fields due to use of agrochemicals (Aridem et al 2014).

Table 4.5 (a): The biodiversity indices for dry season during land preparation

Biodiversity index	Sampling sites									
	Inlet	Plot A	Plot B	Plot D	Plot G	Plot K	Plot L	Plot M	Plot N	Outlet
Shannon-wiener	3.020	3.108	3.132	3.093	3.075	3.080	3.059	3.156	3.134	2.881
Pielou's	0.897	0.923	0.930	0.919	0.913	0.915	0.908	0.937	0.931	0.856
Abundance	121	160	185	178	189	195	181	183	185	70
Taxon richness	25	27	29	29	28	29	28	29	29	22

Note: Plots A, B, D, and G-Fertilizer and Herbicide were used

Plots K, L, M and N- No Fertilizer and Herbicide were used

The diversity indices during land preparation were slightly higher than during growth. The diversity indexes of aquatic organisms obtained in this study were higher than results obtained in rice fields of Southern Mexico (Bond. J. G., et al., 2006) and Southern India (H' = 1.74 – 2.44) (Guarav, Sundaraj, & Karibasvaraj, 2007); but slightly similar to results in rice fields in Punjab Shilvalik, India (H'= 2.98 – 3.02), (Guarav, Sundaraj, & Karibasvaraj, 2007) and Tamil Nadu, India (H' = 2.82 – 3.33, Anbalagan *et al.* 2013 [table 4.5(a), (b) and(c)]

Table 4.5(b): The biodiversity indices for dry season during growth

Biodiversity index	Sampling sites									
	Inlet	Plot A	Plot B	Plot D	Plot G	Plot K	Plot L	Plot M	Plot N	Outlet
Shannon-wiener	3.151	3.099	3.111	3.014	3.117	3.117	3.086	3.127	3.119	3.039
Pielou's	0.936	0.920	0.924	0.895	0.926	0.926	0.916	0.929	0.926	0.903
Abundance	488	461	483	497	487	492	488	515	487	281
Taxon richness	28	29	29	29	29	29	29	29	29	26

Note: Plots A, B, D, and G-Fertilizer and Herbicide were used

Plots K, L, M and N- No Fertilizer and Herbicide were used

Higher diversity indices in Ahero rice paddies could have also been due to the fact that rice cultivation activities within a short rice growing season regulate the abundance and diversity of these organisms (Mogi, 2007) (Asghar, 2010) (Hayasaka, Korenaga, Sanchez, & Goka, 2012). This therefore implies that rice fields are colonized by organisms with short life cycles that are well adapted to the temporary nature of the rice field habitat as earlier suggested by (Heiss, Harp, & Meisch, 1986).

Table 4.5(c): The biodiversity indices for dry season during maturation

Biodiversity index	Sampling sites									
	Inlet	Plot A	Plot B	Plot D	Plot G	Plot K	Plot L	Plot M	Plot N	Outlet
Shannon-wiener	3.117	3.008	3.225	3.145	3.150	3.122	3.126	3.146	3.120	2.990
Pielou's	0.926	0.893	0.958	0.934	0.935	0.927	0.928	0.934	0.927	0.888
Abundance	463	474	515	517	524	495	523	529	517	266
Taxon richness	29	29	28	29	29	29	29	29	29	26

Note: Plots A, B, D, and G-Fertilizer and Herbicide were used

Plots K, L, M and N- No Fertilizer and Herbicide were used

During the wet season the sample sites displayed wide ranges in the measures as shown in Table 4.6(a), (b) and (c). It was observed that taxa richness for B, D, K and N recorded a similar value (29). Shannon Diversity Index and evenness was highest at site D and recording values of 3.361 and 0.998 respectively while the lowest value was at the Outlet recording 2.635 and 0.783. It was also noted that site K and L recorded higher values than most sites for plots where no agrochemicals were applied. The Inlet and Outlet showed remarkable values during growth and maturity with Shannon Diversity values of 3.071 and 3.08 and Pielou's evenness (J) index values of 0.912 and 0.916 for growth and maturity respectively.

Table 4.6(a): The biodiversity indices for wet season during land preparation

Biodiversity index	Sampling sites									
	Inlet	Plot A	Plot B	Plot D	Plot G	Plot K	Plot L	Plot M	Plot N	Outlet
Shannon-wiener	2.964	3.060	3.151	3.361	3.136	3.183	3.087	3.106	3.127	2.635
Pielou's	0.880	0.909	0.936	0.998	0.931	0.945	0.917	0.922	0.928	0.783
Abundance	138	165	215	200	184	157	177	182	177	76
Taxon richness	26	28	29	29	28	29	28	29	29	16

Note: Plots A, B, D, and G-Fertilizer and Herbicide were used

Plots K, L, M and N- No Fertilizer and Herbicide were used

During the wet season among the different stages of the rice crop, there was no significant difference recorded between the biologic metrics evaluated ($p > 0.05$). During the stage of maturation and cut, highest average values were found for the diversity and evenness (2.146 and 0.533, respectively) [Tab. 4.6(a). (b) and (c)]. On the other hand, when compared the different managements of the rice fields with regards to use of agrochemicals (fertilizer and herbicide), a significant difference was verified among the diversity values ($F_{1,4} = 10.98$; $p = 0.03$) and of richness values ($F_{1,4} = 66.26$; $p = 0.002$).

Table 4.6 (b): The biodiversity indices for wet season during growth

Sampling sites

Biodiversity index	Inlet	Plot A	Plot B	Plot D	Plot G	Plot K	Plot L	Plot M	Plot N	Outlet
Shannon-wiener	3.071	3.066	3.147	3.144	3.100	3.051	3.121	3.065	3.290	2.963
Pielou's	0.912	0.911	0.935	0.934	0.921	0.906	0.932	0.910	0.977	0.880
Abundance	426	470	505	519	516	496	523	534	488	272
Taxon richness	27	28	29	29	29	29	29	28	29	25

Note: Plots A, B, D, and G-Fertilizer and Herbicide were used

Plots K, L, M and N- No Fertilizer and Herbicide were used

The management of Inlet and Outlet drainages and the management practices used in the surrounding rice paddies influenced the biological diversity of the rice crops. In the studied plots, the richness and abundance of macroinvertebrates did not change over the rice cultivation cycle. Despite variation of the water depth along the cycle (from 5 to 150cm), the presence of surface water during the entire period in the drainages may be an important factor for the richness and abundance of species (Maltchik L., Rolon, Stenert, Machado, & Rocha, 2011).

Table 4.6(c): The biodiversity indices for wet season during maturation

Biodiversity index	Sampling sites									
	Inlet	Plot A	Plot B	Plot D	Plot G	Plot K	Plot L	Plot M	Plot N	Outlet
Shannon-wiener	3.084	3.096	3.155	3.164	3.123	3.078	3.124	3.102	3.130	2.999
Pielou's	0.916	0.919	0.937	0.940	0.927	0.914	0.928	0.921	0.930	0.891
Abundance	412	452	473	460	495	498	487	516	456	282
Taxon richness	29	29	29	29	29	29	29	29	29	27

Note: Plots A, B, D, and G-Fertilizer and Herbicide were used

Plots K, L, M and N- No Fertilizer and Herbicide were used

Conclusion

The mean values for the macroinvertebrates abundance during the operations varied significantly (One-Way ANOVA at α =0.05, $F_{(5,114)}$ = 100.440, p = 0.000) with the lowest macroinvertebrates mean value (81.3±15.26) recorded during land, nursery and seedbed preparation while the highest macroinvertebrate mean value (245.60 ±40.67) was recorded immediately after fertilizer application at ear formation. Duncan Multiple Range Test (DMRT) further established no significant difference in the mean macroinvertebrate values between the period of land, nursery and seedbed preparations and transplanting. High biodiversity indices were observed in the sampled fields with Shannon Diversity values ranging between 3.059 to 3.16, Pielou's evenness (J) ranged between 0.910 to 0.937 and taxon richness (S) ranging between 27 to 29. Further studies are needed to extend experiments to other paddy rice farming contexts, monitor macroinvertebrate diversity over a longer term in a balanced design, and explore the potential of using such taxa in monitoring soil quality in rice paddies. The studies would identify indicator macroinvertebrate species that are tolerant or intolerant to chemical and physical soil disturbance in the paddies. It would also be necessary to identify any other apparently new macroinvertebrate taxa in paddies that could be using wetlands during some of their growth stages to respond to the changing global climate.

Acknowledgement

We thank the field assistant Mr. Odago from Ahero Rice Research Station for his help in sample collection. We also acknowledge the management and farmers for granting us permission to sample from their rice fields. We are grateful to the departments of botany, fisheries and aquaculture zoology in Maseno University for allowing us use their equipment during field work and the laboratories for sample analysis.

6. Author's contribution

Author 1: Conceptualization, original draft preparation, writing -review and editing, resources, data curation, methodology and analysis.

Author 2: Conceptualization, validation, methodology, review and editing, analysis and supervision.

Author 3: Conceptualization, validation, methodology, review and editing, analysis and supervision.

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International Journal of Academic Management Science Research (IJAMSR)
ISSN: 2643-900X
Vol. 9 Issue 4 April - 2025, Pages: 55-79

APPENDICES

Appendix 1: Sampling sites

Sample Site No.	Block	Coordinates		Altitude	Site characteristic
		Latitudes (S)	Longitudes(E)	(m)	
1	Inlet canal	00.14180	034.93720	1160	Natural env.
2	A1	00.13506	034.95731	1160	Chemical
3	A2	00.13599	034.95558	1155	Chemical
4	B1	00.14270	034.95170	1165	Chemical
5	B2	00.13897	034.95720	1163	Chemical
6	D1	00.14122	034.93780	1158	Chemical
7	D2	00.14135	034.9378	1158	Chemical
8	G1	00.13335	034.94371	1160	Chemical
9	G2	00.13211	034.94638	1162	Chemical
10	K1	00.14984	034.92072	1156	No chemical
11	K2	00.14875	034.92353	1157	No chemical
12	L1	00.14875	0034.92211	1156	No chemical
13	L2				No chemical
14	M1	00.15177	034.91806	1155	No chemical
15	M2				No chemical
16	N1	00.15165	034.91889	1152	No chemical
17	N2	00.15151	034.92133	1154	No chemical
18	Outlet Canal	00.13578	034.95564	1153	Natural env.

Appendix 2: Total Macroinvertebrate taxa collected in the rice fields during dry & wet season

Phylum	Class	Order	Family	Dry Season	Wet Season	TOTAL
Arthropoda	Insecta	Odonata	Gomphidae	70	52	122
Arthropoda	Insecta	Odonata	Libellulidae	237	206	443
Arthropoda	Insecta	Odonata	Coanagrionidae	168	170	338
Arthropoda	Insecta	Odonata	Aeshnidae	130	124	254
Arthropoda	Insecta	Ephemeroptera	Baetidae	400	436	836
Arthropoda	Insecta	Ephemeroptera	Caenidae	386	408	794

Arthropoda	Insecta	Coleoptera	Dytistidae	550	578	1128
Arthropoda	Insecta	Coleoptera	Gyrinidae	832	882	1714
Arthropoda	Insecta	Coleoptera	Hydrophilidae	534	525	1059
Arthropoda	Insecta	Coleoptera	Noteridae	354	311	665
Arthropoda	Insecta	Coleoptera	Haliplidae	308	284	592
Arthropoda	Insecta	Diptera	Tipulidae	488	440	928
Arthropoda	Insecta	Diptera	Chironomidae	963	1223	2186
Arthropoda	Insecta	Diptera	Culicidae	717	930	1647
Arthropoda	Insecta	Diptera	Tabanidae	230	208	438
Arthropoda	Insecta	Hemiptera	Belostomatidae	788	855	1643
Arthropoda	Insecta	Hemiptera	Corixidae	620	542	1162
Arthropoda	Insecta	Hemiptera	Gerridae	358	360	718
Arthropoda	Insecta	Hemiptera	Nepidae	411	420	831
Arthropoda	Insecta	Hemiptera	Notonectidae	502	482	984
Arthropoda	Insecta	Hemiptera	Veliidae	166	128	294
Arthropoda	Arachnida	Acari	Acaricidae	424	426	850
Arthropoda	Arachnida	Acari	Hydrachnidae	374	376	750
Annelida	Oligochaeta	Naidomorpha	Tubificidae	215	265	480
Annelida	Clitellata	Huridinae	Huridae	125	91	216
Mollusca	Gastropoda	Prosobrancha	Hydrobiidae	278	468	746
Mollusca	Gastropoda	Prosobrancha	Physidae	150	159	309
Mollusca	Gastropoda	Prosobrancha	Thiaridae	97	101	198
Mollusca	Bivalvia	Sphaeriidae	Unionidae	44	45	89
				10919	11495	

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Appendix 3: Macroinvertebrate abundance per activity during dry season

Family	8/10/2021	22/10/2021	5/11/2021	19/11/2021	3/12/2021	17/12/2021	Total
Gomphidae	2	6	15	11	16	20	70
Libellulidae	10	12	40	50	55	70	237
Coanagrionidae	6	12	30	42	36	42	168
Aeshnidae	6	12	22	26	31	33	130
Baetidae	5	9	160	180	20	26	400
Caenidae	4	8	165	179	20	10	386
Dytistidae	27	51	100	100	135	137	550
Gyrinidae	62	68	157	183	180	182	832
Hydrophilidae	34	38	80	98	138	146	534
Noteridae	20	36	69	85	70	74	354
Haliplidae	13	21	60	74	63	77	308
Tipulidae	21	43	98	116	101	109	488
Chironomidae	65	86	196	208	200	208	963
Culicidae	48	54	150	164	148	153	717
Tabanidae	8	12	48	60	58	44	230

International Journal of Academic Management Science Research (IJAMSR)
ISSN: 2643-900X
Vol. 9 Issue 4 April - 2025, Pages: 55-79

Belostomatidae	63	71	138	150	186	180	788
Corixidae	49	59	105	109	143	155	620
Gerridae	29	33	60	64	84	88	358
Nepidae	33	37	69	75	96	101	411
Notonectidae	40	44	88	96	120	114	502
Veliidae	18	20	24	28	39	37	166
Acaricidae	32	20	100	108	79	85	424
Hydrachnidae	40	36	93	101	55	49	374
Tubificidae	20	45	34	38	38	40	215
Hurididae	7	24	20	22	25	27	125
Hydrobiidae	21	11	27	23	106	90	278
Physidae	15	25	17	15	37	41	150
Thiaridae	9	17	10	9	27	25	97
Unionidae	4	9	6	7	8	10	44
	711	919	2181	2421	2314	2373	

Appendix 4 : Total Macroinvertbrate (individual family) abundance per activity during wet season

Family	11/3/2022	25/3/2022	8/4/2022	22/4/2022	6/5/2022	20/5/2022	Total
Gomphidae	4	6	9	11	10	12	52
Libellulidae	12	16	43	45	45	45	206
Coanagrionidae	12	14	26	32	44	42	170
Aeshnidae	20	9	22	25	26	22	124
Baetidae	12	14	180	192	20	18	436
Caenidae	11	12	178	171	24	12	408
Dytistidae	52	64	94	104	137	127	578
Gyrinidae	73	81	180	184	184	180	882
Hydrophilidae	39	45	92	103	126	120	525
Noteridae	22	25	63	67	69	65	311
Haliplidae	17	19	58	60	68	62	284
Tipulidae	32	34	97	101	92	84	440
Chironomidae	109	118	260	262	240	234	1223
Culicidae	90	95	190	195	183	177	930
Tabanidae	12	12	47	51	49	37	208
Belostomatidae	78	82	152	145	202	196	855
Corixidae	37	39	98	102	130	136	542
Gerridae	24	26	64	68	90	88	360
Nepidae	31	33	74	78	101	103	420
Notonectidae	34	38	83	89	120	118	482
Veliidae	11	13	22	24	30	28	128
Acaricidae	38	34	110	114	68	62	426
Hydrachnidae	44	46	95	97	49	45	376

Tubificidae	21	47	43	49	49	56	265
Hurididae	7	8	21	20	19	16	91
Hydrobiidae	36	47	59	57	132	137	468
Physidae	13	15	18	19	45	49	159
Thiaridae	11	10	13	11	27	29	101
Unionidae	5	5	5	8	10	12	45
	907	1007	2396	2484	2389	2312	