Using Benthic Macroinvertebrate Distribution and Water Quality as Organic Pollution Indicators for Fish Farming Areas in Rawang Sub-basin, Selangor River, Malaysia: A Correlation Analysis

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ABSTRACT

Fish farming activities are essential to the economy of many countries. However, the discharge of fish farm effluents into nearby rivers can negatively impact benthic macroinvertebrates and water quality. In Malaysia, the correlation between water quality and benthic macroinvertebrates in areas impacted by fish farming has not been discussed comprehensively. Hence, this research investigated the connection between benthic communities and water quality in the Rawang sub-basin of the Selangor River using several statistical methods. Based on ease of accessibility and proximity to freshwater fish farms, and by using a random sampling method, seven sampling sites in six rivers were chosen including one reference site. Sampling of benthic macroinvertebrates and river water was carried out between April 2019 and March 2020. Principal Component Analysis (PCA) revealed that fish farming operations influence various water quality parameters such as dissolved oxygen (DO), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), pH, and ammonia-nitrogen. Canonical Correspondence Analysis (CCA) revealed that families Aeolosomatidae, Chironomidae, Lumbriculidae, Naididae, Planorbidae, and Tubificidae are tolerant to organic pollution. Their abundance was correlated with high BOD_5, COD, turbidity, TSS, and ammonia-nitrogen. On the other hand, the families Caenidae, Gomphidae, Aytidae, Leptophlebiidae, Thiaridae, and Viviparidae are sensitive to organic pollution and were correlated with DO concentration. This research revealed that the correlation between benthic macroinvertebrate communities and water quality in the area is affected explicitly by fish farms.

Keywords: Aquaculture, Bioindicator, Biotic index, Multivariate statistical analyses, Water pollution

INTRODUCTION

A river system is a vital natural entity that provides critical water resources for numerous ecosystem functions. Rivers are used for various purposes in day-to-day life; however, water pollution increases due to these anthropogenic activities (Liyanage and Yamada, 2017). Among the more detrimental activities, the discharge of effluents from fish farming operations into adjacent rivers and streams without appropriate treatment affects the water quality and aquatic biota (Bhavsar *et al*., 2016). Traditionally, physicochemical parameters have been used to assess river water quality despite their determination being commonly cost-intensive, time-consuming, and dependent on the particular

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instruments used. Similarly, physicochemical parameters fluctuate over time and only reflect environmental conditions at the moment of measurement (Aazami *et al*., 2015). Therefore, biomonitoring is currently being used to evaluate river water quality using benthic macroinvertebrates in tropical countries, including Colombia, Thailand, Singapore, Philippines (Morse *et al*., 2007; Musonge *et al*., 2020) and Malaysia (Azis and Abas, 2021).

Benthic macroinvertebrates are invertebrate organisms visible to the naked eye that live in, on or near the bottom sediments of aquatic water bodies (Aweng *et al*., 2012). They are a primary food source of fishes and other predators, meaning they are essential in the food web (Muro-Torres *et al*., 2020). Also, they play a vital role in any aquatic community because of their ability to promote mineralization, mixing of sediments, oxygen flux into sediments, cycling of organic matter. Furthermore, they can aid in assessing inland water quality (Sharma *et al*., 2013). Benthic macroinvertebrates are the most reliable, accepted, commonly used, and cost-effective tools for biomonitoring (Ghosh and Biswas, 2015; Selvanayagam and Abril, 2016) due to their high biodiversity, minimal mobility, relatively long life cycle, bottom-dwelling nature, and high degree of sensitivity towards changes in their environment (Deborde *et al*., 2016; Selvanayagam and Abril, 2016). As such, benthic macroinvertebrates are commonly divided into three different ecological groups according to their pollution tolerance levels: sensitive, moderately tolerant, and tolerant (Lewis, 2016).

There are recent reports on the relationship between benthic macroinvertebrates and water quality in natural water bodies of tropical counties such as Thailand (Sirisinthuwanich *et al*., 2017), India (Barman and Gupta, 2015), Malaysia (Shafie *et al*., 2017), and Taiwan (Narangarvuu *et al*., 2014). However, none of them specifically focused on areas affected by fish farms. In addition, the effect of land-use changes on benthic macroinvertebrates (Ling, 2010; Rak *et al*., 2014; Hasmi *et al*., 2017) and water quality (Al-badaii *et al*., 2013; Tan and Rohasliney, 2013; Toriman *et al*., 2018) in Malaysian rivers has been extensively researched. However, the impact of fish farming operations on benthic macroinvertebrates in rivers is not acknowledged. Hence, in this study, some of the unique characteristics of benthic macroinvertebrates were applied to assess the disturbance effect from fish farming on the stream ecology. The initial aim was to characterize the water quality of areas affected by fish farms. The second aim was to ascertain the correlation between benthic communities and water quality in the Rawang sub-basin of the Selangor River. The last aim was to identify the most abundant pollution-tolerant benthic macroinvertebrates that are present in areas affected by fish farming activities.

MATERIALS AND METHODS

The study area

The sampling area for the current study was the Rawang sub-basin, one of ten sub-basins in Malaysia's Selangor River basin (Chowdhury *et al*., 2018). This sub-basin has a tropical climate, with a maximum daytime temperature of 33.4 °C and a minimum nighttime temperature of 22.8 °C, and average annual relative humidity ranging from 78 % to 87 % (Seyam and Othman, 2015). Yearly rainfall averages between 2,000 and 3,000 mm. This area is subjected to a rainy season from December to March, intermediate seasons from April to May and September to November, and a dry season from June to August (Awang, 2015).

The seven sampling sites were located in the Rawang sub-basin (Figure 1) on the Guntong River (SR1) and its tributary (SR2), the Kuang River (SR3 and SR7), the Gong River (SR4), the Buaya River (SR5), and the Serendah River (SR6). The Guntong tributary (SR2) was chosen as a reference site based on these characteristics: no upstream fish farms, minimal natural damage to its surroundings, and absence of human disturbance. All selected sampling sites were located close to the riverbank. Due to accessibility, the sampling sites were located at varying distances (in parentheses) downstream from a fish farm effluent point: SR1 and SR3 (200 m), SR4 and SR5 (20 m), SR6 (400 m), and SR7 (400 m). However, there were no points of wastewater discharge from other sources between the sampling sites and the fish farm discharge points. All of the fish farms in this sub-basin are land-based and are utilized for aquaculture (SR1, SR3, and SR6) or sport fishing (SR4, SR5, and SR7). Fish farms are named (e.g., Farm1) by their respective sampling site number (Table 1).

Table 1. Description of sampling sites.

Sampling site	Fish farm	Fish farm type	Proximity to fish farm (approx.)	Stream order	Riverbank substrates (dominant)
Guntong River (SR1)	Farm1	Aquaculture	200 m	4th order	Sand and silt
Guntong tributary (SR2) /Reference site	No upstream fish farms.	-	-	3rd order	Sand, silt and rock
Kuang River (SR3)	Farm3	Aquaculture	200 m	3rd order	Sand, silt and rock
Gong River (SR4)	Farm4	Sport fishing	20 m	3rd order	Sand and silt
Buaya River (SR5)	Farm5	Sport fishing	20 m	4th order	Sand, silt and rock
Serendah River (SR6)	Farm6	Aquaculture	400 m	3rd order	Sand and rock
Kuang River (SR7)	Farm7	Sport fishing	400 m	3rd order	Sand, silt and rock

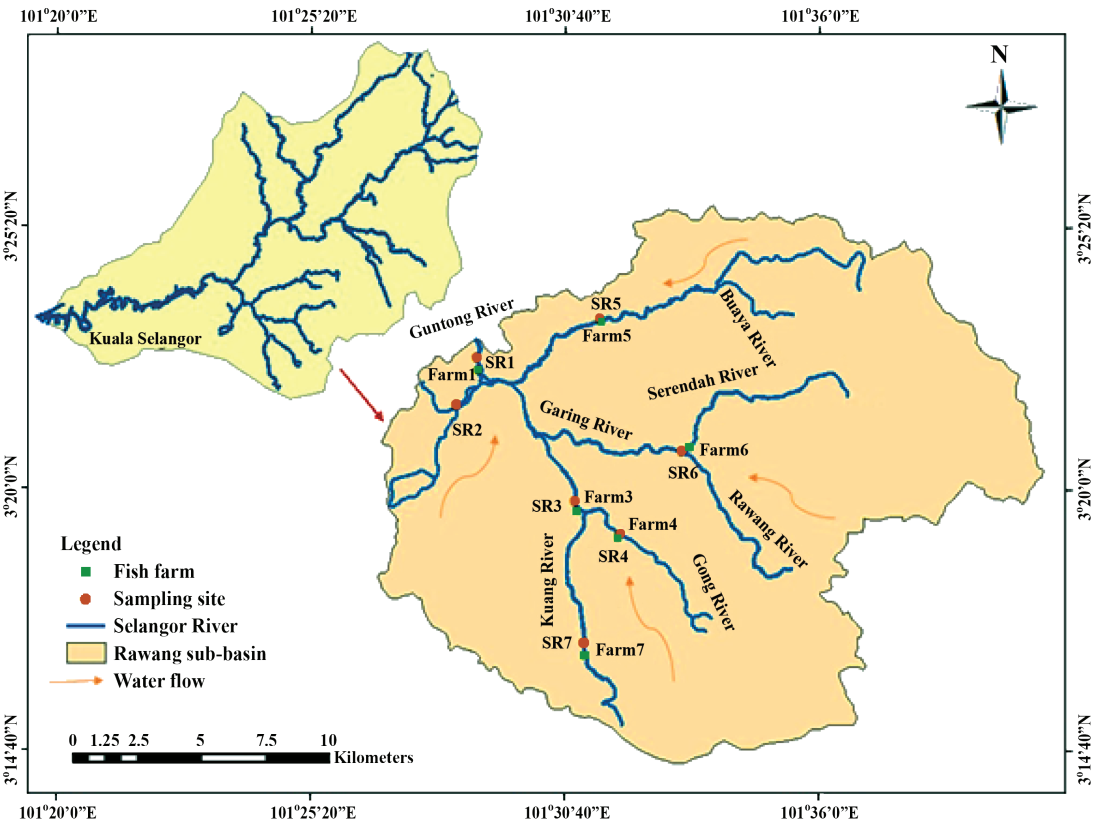

Figure 1. Locations of sampling sites (SR1 to SR7) and fish farms in the study area.

Water quality analysis

Water and macroinvertebrate samples were collected every other month from the selected sampling sites from April 2019 to February 2020, and once in March 2020. An additional sampling trip was made in March 2020 to allow an appropriate rarefaction curve for benthic macroinvertebrates (Total N = 147 samples/sub-basin = 7 sites×7 sampling trips×3 replicates). Water temperature (WT) (Thermo Scientific Orion 3-Star, USA), turbidity (HACH 2100 P, USA), pH (Thermo Scientific Orion 3-Star, Indonesia), and dissolved oxygen (DO) (YSI 52, USA) of the water samples were all monitored *in-situ* using portable meters. During transit to the laboratory for further examination, all collected water samples were maintained at 4 °C.

Total suspended solids (TSS) were quantified in the laboratory using the gravimetric technique (APHA, 2012). TSS and turbidity were analysed in this study because they can influence the river water due to the release of uneaten foods, fish pellets, fish excrement, leftover feed, and organic food sludge from fish farms (Fadaeifard *et al*., 2012). The ammonia-nitrogen and chemical oxygen demand (COD) concentrations were measured using a UV spectrophotometer, utilizing the salicylate technique and reactor digestion method, respectively (DR 2800, HACH, Germany). Prior to biochemical oxygen demand (BOD_5) analysis, all water samples were kept in dark-colored glass bottles at room temperature. The initial BOD_5 concentration was determined using a BOD probe meter (YSI 5905, USA). For five days, samples were stored in an incubator at temperatures below 20 °C. After the 5-day incubation period, the BOD content of the water samples was rechecked. The difference between measurements for each sample was used to determine the BOD_5.

Analysis of benthic macroinvertebrates

Benthic macroinvertebrate sampling was performed every other month from April 2019 to February 2020. An additional sampling trip was made in March 2020 to allow an appropriate rarefaction curve. Three sampling gears were employed to gather the samples: a kick net, a D-frame dip net, and a hand scoop (Total N = 735 samples/sub-basin = 7 sites×7 sampling trips×3 gears×5 replicates). The D-frame dip net had a long handle fitted with a cone-shaped, 0.3 m diameter net with 300 µm mesh. Kick net has the net frame size of 390 mm×320 mm with 500 µm as the mesh size. Both the D-frame aquatic kick net and the D-frame dip net were used to capture benthic macroinvertebrates against the water current. A one-square-meter patch of the substrate was disturbed for at least two or three minutes in front of the net via the kick-sampling technique (Merritt and Cummins, 1996). Samples of sediments were collected using a hand scooping method to a depth of 5 cm below the river bottom. Samples were poured into polyethylene bags and preserved using 70% ethanol, and then transported for further analysis at the Aquatic Laboratory of the Faculty of Forestry and Environment, Universiti Putra Malaysia (UPM).

Five replicates of samples collected by each gear were composited into a single sample in the laboratory. The samples were cleaned and sorted using a 0.5 mm sieve (APHA, 2012). Then, the benthic macroinvertebrates were stored in 70% ethanol for subsequent examination. Oligochaete worms and chironomid larvae were mounted on temporarily prepared glass sides and identified using a compound microscope. Under a dissecting microscope, more macroinvertebrate taxa were discovered. All benthic macroinvertebrates were further identified to their closest taxonomic unit using references from Brinkhurst (1971), Brinkhurst and Jamieson (1971), Hong (1994), Zhao (1994), Merritt and Cummins (1996), Yong and Yule (2004), Sangpradub and Boonsoong (2006), Thorp and Lovell (2014). However, the Cladocera were not identified to family level due to a lack of standard references.

Relative abundance

In order to determine the dominant taxa of the study area and seasonal occurrences, the relative abundance of each taxonomic group was calculated.

Data analysis

Microsoft Office EXCEL 2010 was used to create all descriptive statistics. The comparison of means was performed using a one-way ANOVA test facilitated by the IBM SPSS Statistics 25 software to determine the sites with the highest and lowest water quality. Principal Component Analysis (PCA) was subsequently applied using the IBM SPSS Statistics 25 software to characterize the water quality of the areas affected by fish farms. The Kaiser-Meyer-Olkin (KMO) and Bartlett's sphericity tests were used to determine the sample suitability for identifying data structure. Because only one individual from Cladocera, Dytiscidae, and Ephydridae was sampled during the study period, these three taxa were excluded from the data analysis. The exclusion was done to reduce analysis errors and prevent bias. The Pearson correlation was used to determine the correlation between the relative abundance of benthic macroinvertebrates and water quality parameters. Canonical Correspondence Analysis (CCA) was also performed using the CANOCO program (version 4.5) to ascertain the correlation of water quality parameters between benthic macroinvertebrates and sampling sites. CCA was used to log-transform the abundance of benthic macroinvertebrates. CanoDraw for Windows was used to construct a triplot ordination diagram using water quality, benthic macroinvertebrate, and sampling site data.

RESULTS AND DISCUSSION

Water quality

Table 2 presents the mean values for selected water quality parameters in the Rawang sub-basin of the Selangor River. Gong River (SR4) (2.78±1.65 $mg{\cdot}L^{-1}$) produced the lowest DO concentration, whereas DO was highest at the reference site (SR2)

Table 2. Summary of water quality parameters (mean±SD) among sites and results of statistical comparisons.

Water quality parameter	SR1	SR2	SR3	SR4	SR5	SR6	SR7	p value	F Value
Water temperature (°C)	28.26±1.17	26.32±1.41	28.67±1.43	30.18±1.48	27.01±0.94	28.48±1.63	27.14±1.46	0.00	18.580
DO ($mg{\cdot}L^{-1}$)	5.01±1.33	6.43±0.50	4.54±1.15	2.78±1.65	5.98±1.05	6.11±0.66	5.58±0.77	0.00	28.692
pH	6.40±0.23	6.35±0.30	6.61±0.20	6.57±0.17	6.15±0.66	6.47±0.33	6.66±0.83	0.07	3.130
Turbidity (NTU)	129.60±50.71	12.14±3.12	131.73±86.94	119.67±92.53	116.41±222.27	103.96±89.51	145.84±119.33	0.05	3.219
BOD_5 ($mg{\cdot}L^{-1}$)	3.78±1.19	0.54±0.25	4.74 ±1.00	6.50±1.27	3.24±0.71	2.17±0.57	2.85±0.68	0.00	97.620
COD ($mg{\cdot}L^{-1}$)	33.14±6.05	0.05±0.22	33.10±5.58	45.24±7.25	27.76±5.69	23.14±6.08	23.00±5.03	0.00	132.671
Ammonia-Nitrogen ($mg{\cdot}L^{-1}$)	1.08±0.33	0.16±0.25	1.93±1.19	1.86±0.60	1.45±0.78	0.39±0.34	1.37±0.95	0.00	19.335
TSS ($mg{\cdot}L^{-1}$)	91.12±36.73	21.35±27.39	99.49±56.54	73.42±54.71	106.40±179.44	68.72±69.68	101.25±81.08	0.12	2.850

Note: DO = Dissolved Oxygen; NTU = Nephelometric Turbidity Unit; BOD_5 = five-day Biochemical Oxygen Demand; COD = Chemical Oxygen Demand; TSS = Total Suspended Solids

(6.43±0.50 mg·L^{-1}). The mean BOD_5 concentration ranged from 0.54±0.25 mg·L^{-1} to 6.50±1.27 mg·L^{-1} across all sites. The COD concentration showed considerable variation among sites. It ranged from 0.05±0.22 mg·L^{-1} at the reference site (SR2) to 45.24±7.25 mg·L^{-1} at Gong River (SR4), while ammonia-nitrogen concentration fluctuated less among sites (from 0.16±0.25 mg·L^{-1} at the reference site [SR2] to 1.93±1.19 mg·L^{-1} at Kuang River [SR3]). The results also reveal that water quality at SR6 differs from the other sampling sites when excluding the reference sampling site (SR2).

The PCA correlation matrix showed that several water quality parameters, including water temperature, DO, pH, ammonia-nitrogen, BOD_5, COD, turbidity, and TSS, have coefficient values of 0.3 or greater. The Chi-square ($\chi 2$) of Bartlett's and KMO test results were 835.596 and 0.674, respectively, at a significance level of $p<0.05$. The KMO value surpassed the 0.6 criteria, indicating the dataset is suitable for PCA. The first, second, and third principal components had eigenvalues greater than one, and accounted for 43.108 %, 22.234 %, and 13.675 % (total of 79.017 %) of the total variance, thus classifying the data into three main components (Table 3).

The varimax rotated component matrix was loaded for each water quality parameter (Table 3). In general, component loadings higher than 0.6 are used to evaluate the extracted components in a PCA (Tashtoush, 2015). Typically, component loading levels of more than 0.75, between 0.75 and 0.50, and between 0.50 and 0.30 are considered high, moderate, and weak, respectively (Liu *et al*., 2003).

The first principal component is notable for strong positive loadings of BOD_5, and COD, while a moderate positive loading of ammonia-nitrogen was also observed (Table 3). Furthermore, DO contributes a significant negative loading on the first principal component, suggesting that fish farming resulted in organic pollution in the study area. Similarly, significant DO, BOD_5, and COD loading were linked with a large amount of organic waste (Tan and Beh, 2016), resulting from the regular discharge of fish farm wastes into adjacent rivers (Minoo *et al*., 2016). Consequently, these indicators suggest a high level of human activity. Conversely, the positive loading of ammonia-nitrogen on the first principal component (Table 3) indicated organic contamination, as ammonia-nitrogen is a waste product of fish metabolism and originates from fish feed constituents.

Table 3. Principal components (PC) and varimax rotated component matrix.

	Eigenvalue explained by PCs		
	3.449	1.779	1.094
	Percentage of total variance explained		
	43.108	22.234	13.675
	Component matrix		
Variables	**PC1**	**PC2**	**PC3**
Water Temperature	0.576	-0.036	0.421
pH	0.070	0.057	**0.849**
DO	**-0.852**	-0.129	-0.113
Turbidity	0.126	**0.970**	0.074
Ammonia-N	**0.647**	-0.051	-0.455
BOD_5	**0.949**	0.137	0.025
COD	**0.908**	0.155	0.042
TSS	0.086	**0.979**	0.003

In contrast, the second principal component (Table 3) had significant positive loadings of TSS and turbidity, indicating a significant physical disturbance and organic pollution from fish farming in the study area. Turbidity measures the concentration of suspended solids in the river water. Therefore, the fish harvest might generate a substantial polluting load that would produce a higher amount of TSS in the receiving water (Pajooh *et al*., 2016). Additionally, the TSS of the wastewater is often higher than that of the intake water, through the presence of colloid particles, organometallic compounds, fish excrement, leftover feed, and organic food sludge (Fadaeifard *et al*., 2012), also creating increased turbidity.

The third PC, with an eigenvalue of 1.094 and explaining 13.67 % of the total variance, had a strong positive loading of pH (Table 3). The anaerobic conditions in the river (indicated by the strong loading of dissolved organic matter) enabled the formation of organic acids. Moreover, ammonia-nitrogen, nitrate, and phosphate also contain ionic components. These ionic components are released due to organic pollution. Therefore, changes in pH can affect the productivity of fish farming activities with ionic contamination of river water, indicating organic pollution.

Benthic macroinvertebrates and water quality parameters

Within the research area, 7,677 benthic macroinvertebrates from 27 families were collected. From these families, 35 benthic macroinvertebrates were identified up to the genus level. Specimens of Tubificidae, Haplotaxidae, Viviparidae, Lumbriculidae, Atyidae, and Planorbidae were identified to family, while Tanypodinae was identified at the subfamily level, and specimens of Cladocera and one Oligochaeta were only identified to Order. Hettige *et al*. (2020) published these amalgamated data. Tubificidae was the most dominant family (36.80 %), with Chironomidae as the second-most dominant (28.85 %), followed by Naididae (15.94 %) (Figure 2).

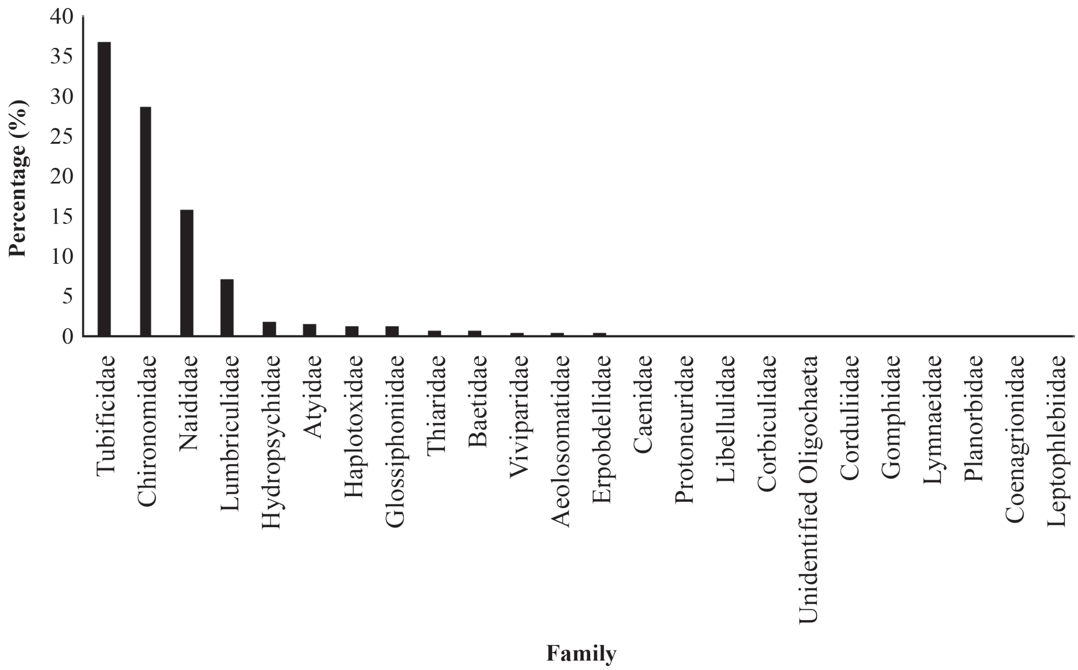

Figure 2. Relative abundance of benthic macroinvertebrate families recorded at all sampling sites in the Rawang sub-basin.

Table 4 portrays the seasonal variation of total and relative abundance of benthic macroinvertebrate taxa during the study period. Of the dominant taxa, the relative abundance of Chironomidae was notably high in August and low in April 2019 (Table 4). In August (usually a drymonth), the high amount of organic matter due to fish farming effluents favored chironomid survival. They were also present in all the sampling months during the study period, implying that they survive seasonal changes. Akindele and Liadi (2013) reported a similar finding in the Aiba Stream, Iwo, Southwestern Nigeria.

Compared to the all the sampling months, higher relative abundance was in April 2019 and lower relative abundance was in February 2020. Also, the highest Tubificidae density was found due to increased aggregation of individuals during the dry and intermediate seasons (Martins *et al*., 2008). The number of individuals in February was reduced due to the washout of sediments and dilution. Theoretically, Malaysia especially the Selangor River receives approximately 98 mm maximum precipitation in February 2020 that could potentially create a heavy flow (~ 69 $m^3 \cdot s^{-1}$) that can

Table 4. Seasonal variation of total and relative abundance of benthic macroinvertebrate taxa during the study period.

Family	2020										2021			
	April		June		August		October		December		February		March	
	TA	RA%	TA	RA%	TA	RA%	TA	RA%	TA	RA%	TA	RA%	TA	RA%
Aeolosomatidae	-	0.00	7	1.42	1	0.08	9	0.82	12	1.95	16	1.03	-	0.00
Atyidae	21	2.35	23	4.66	50	4.13	15	1.37	1	0.16	11	0.71	3	0.16
Baetidae	-	0.00	-	0.00	-	0.00	-	0.00	7	1.14	1	0.06	55	3.02
Caenidae	1	0.11	-	0.00	6	0.50	-	0.00	1	0.16	6	0.39	6	0.33
Chironomidae	24	2.68	120	24.29	578	47.77	147	13.44	135	21.99	357	23.06	753	41.33
Corbiculidae	1	0.11	-	0.00	-	0.00	2	0.18	1	0.16	6	0.39	1	0.05
Coenagrionidae	-	0.00	1	0.20	2	0.17	-	0.00	-	0.00	-	0.00	-	0.00
Corduliidae	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00	-	0.00	3	0.16
Erpobdellidae	29	3.24	5	1.01	-	0.00	1	0.09	1	0.16	-	0.00	5	0.27
Glossiphoniidae	-	0.00	2	0.40	43	3.55	34	3.11	23	3.75	1	0.06	3	0.16
Gomphidae	-	0.00	-	0.00	3	0.25	-	0.00	-	0.00	1	0.06	1	0.05
Haplotoxidae	-	0.00	9	1.82	4	0.33	23	2.10	20	3.26	44	2.84	8	0.44
Hydropsychidae	-	0.00	-	0.00	-	0.00	12	0.10	54	8.79	20	1.29	68	3.73
Leptophlebiidae	-	0.00	-	0.00	2	0.17	-	0.00	-	0.00	1	0.06	-	0.00
Libellulidae	1	0.11	-	0.00	17	1.40	-	0.00	1	0.16	5	0.32	-	0.00
Lumbriculidae	-	0.00	84	17.00	77	6.36	34	3.11	29	4.72	146	9.43	157	8.62
Lymnaeidae	1	0.11	-	0.00	1	0.08	-	0.00	-	0.00	1	0.06	1	0.05
Naididae	-	0.00	43	8.70	49	4.05	216	19.74	108	17.59	538	34.75	269	14.76
Planorbidae	-	0.00	-	0.00	1	0.08	3	0.27	-	0.00	-	0.00	-	0.00
Protoneuridae	-	0.00	-	0.00	10	0.83	2	0.18	-	0.00	1	0.06	6	0.33
Thiaridae	16	1.79	3	0.61	14	1.16	5	0.46	8	1.30	18	1.16	4	0.22
Tubificidae	789	88.26	184	37.25	340	28.10	577	52.74	163	26.55	360	23.26	441	24.20
Unidentified Oligochaeta	-	0.00	-	0.00	-	0.00	5	0.46	4	0.65	1	0.06	1	0.05
Viviparidae	9	1.01	10	2.02	11	0.91	7	0.64	1	0.16	7	0.45	-	0.00

Note: TA = Total Abundance; RA = Relative Abundance destroy the microhabitat of the macroinvertebrates during high rains (Hairan *et al*., 2021). During the rainy season, high flows typically occur. These can impact aquatic macroinvertebrates directly and indirectly through habitat changes. Moreover, the highest relative abundance of Naididae was recorded in December 2019 (rainy season), and the lowest relative abundance of Naididae was recorded in April 2019 (dry season). Timm (2020) demonstrates that the reproductive capacity of Naididae decreases at elevated temperatures in the dry season (i.e., 25 °C to 35 °C). Therefore, the reproductive capacity based on temperature could be a reason for the high abundance of Naididae during the rainy months.

Among the Ephemeroptera, the highest relative abundance of Baetidae was observed in December 2019. A high abundance of Caenidae was noted from December 2019 to March 2020 due to improved water quality through organic waste reduction. It facilitated more suitable conditions for the survival of Ephemeroptera. Suhaila *et al*. (2014) reported similar findings in rivers in Gunung Jerai Forest Reserve, Malaysia. There was no remarkable temporal variation of Leptophlebiidae during the study period because no individuals of Leptophlebiidae were recorded in many months. However, the highest relative abundance of Leptophlebiidae was recorded in August 2019. Another dominant group was Atyidae, which was found as dominant during June and August 2019. Many researchers have recorded higher densities of Atyidae during the dry period (Tchakonte *et al*., 2014). Therefore, this family is not suitable as an indicator due to inconsistent behavior seasonally.

Among the taxa that are moderately tolerant to pollution, the highest relative abundance of Libellulidae was recorded in August 2019, whereas none were recorded in June, October 2019, and March 2020. The second highest value was recorded in February 2020. Similarly, the highest relative abundance of Protoneuridae was recorded in August 2019. Typically, the Odonata diversity is higher during the rainy season than the dry season due to reduced water levels in the river during the dry season, which may cause decreased food resources for Odonata. Likewise, Chi *et al*. (2017) reported that the availability of niches and resources (biotic and abiotic) during the rainy season allows taxa to co-exist with more diverse ecological requirements and thus results in a higher number of taxa. Therefore, the present findings concur with Chi *et al*. (2017).

The correlation coefficient values between water quality and relative abundance of benthic macroinvertebrates families are provided in Table 5. The relative abundance of Aeolosomatidae had a strong negative correlation ($r = -0.788$; $p<0.05$) with the pH of river water impacted by fish farming activities (Table 5). Likewise, relative abundance of Lumbriculidae had a strong negative correlation with COD ($r = -0.889$; $p<0.05$) and turbidity ($r = -0.782$; $p<0.05$) but was positively correlated with BOD_5 ($r = 0.769$; $p<0.01$). Therefore, higher organic pollution discharged from the fish farm will elevate the concentration of the BOD, thus showing a higher presence of Lumbriculidae. Hettige *et al*. (2020) presented a high number of Lumbriculidae in SR1, SR3, and SR5 where the fish farm discharge point was in close proximity to the sampling sites. Similarly, Medupin *et al*. (2020) identified a positive correlation between BOD and Lumbriculidae in the urban river Medlock, northwest of England, UK. The urban river is organically polluted due to anthropogenic activities. The Lumbriculidae abundance may indicate organic contamination, as this family is known to have a strong correlation with organic enrichment.

Moreover, relative abundance of Planorbidae showed strong correlation with water temperature ($r = 0.811$; $p<0.05$), DO ($r = -0.928$; $p<0.05$), and BOD_5 ($r = 0.817$; $p<0.01$). Likewise, Sharma *et al*. (2013) found a negative correlation with DO and a positive correlation with BOD for Planorbidae in Gho-Manhasan stream, Jammu, which was impacted by human activities. This is because some Planorbidae can survive in low oxygen concentrations. Also, high input of organic matter in effluents may facilitate an ideal habitat for Planorbidae in fish farming-impacted rivers. Furthermore, strong negative correlations were noted between Viviparidae and turbidity ($r = -0.811$; $p<0.05$) and TSS ($r = -0.802$; $p<0.05$) (Table 5) at sites specifically affected by fish farms. On the contrary, high abundance of Viviparidae has been reported in low turbidity and TSS in natural waters

(Salmiati *et al*., 2017; Imroatushshoolikhah *et al*., 2021). This has made Viviparidae to normally be considered a "pollution sensitive" taxon.

Figure 3 presents the CCA triplot, with the first and second axes having eigenvalues of 0.278 and 0.178, respectively. These two are essential axes because they accurately represent the more significant percentage of family variation observed in the current research and their relationships with water quality parameters. For the x-axis, CCA selected TSS, COD, turbidity, BOD_5, ammonia-nitrogen, pH, and DO. The y-axis represents water temperature. Although the third and fourth axes had relatively low eigenvalues (0.075 and 0.055), the first and second axes explained 43.6 % and 28 % of the variation, respectively. However, the third and fourth axes correlations with families of benthic macroinvertebrates and water quality parameters were only 11.8 % and 8.6 %, respectively.

Group A was prominent in the CCA triplot, and consisted of several families (Figure 3). Aeolosomatidae, Chironomidae, Lumbriculidae, Naididae, Planorbidae, and Tubificidae were positively related to a high concentration of TSS, turbidity, ammonia-nitrogen, BOD_5, COD, and water temperature, but negatively associated with

Table 5. Relationship (presented by correlation coefficients, r) between the relative abundance of benthic macroinvertebrate taxa and physico-chemical parameters.

Parameter	WT	DO	pH	BOD_5	COD	TSS	Turbidity	Ammonia-N
Aeolosomatidae	-0.340	0.370	-0.788*	-0.344	-0.352	-0.356	-0.560	-0.349
Atyidae	-0.107	0.002	0.064	-0.175	-0.371	-0.546	-0.575	-0.185
Baetidae	-0.420	0.241	0.212	-0.148	-0.097	0.454	0.398	0.180
Caenidae	0.063	0.169	0.437	0.009	0.109	0.499	0.492	0.206
Chironomidae	-0.222	0.159	0.474	-0.102	-0.194	0.220	0.144	0.252
Corbiculidae	0.377	0.037	-0.147	0.042	0.248	0.029	0.157	-0.253
Coenagrionidae	-0.320	0.194	0.042	-0.335	-0.550	-0.528	-0.633	-0.203
Corduliidae	-0.296	0.132	0.504	-0.129	-0.111	0.313	0.370	0.124
Erpobdellidae	0.072	-0.075	-0.129	0.106	0.232	0.218	0.260	-0.020
Glossiphoniidae	0.330	-0.475	-0.150	0.613	0.504	0.391	0.240	0.736
Gomphidae	0.212	-0.134	0.503	0.224	0.167	0.318	0.304	0.402
Haplotoxidae	-0.059	0.223	-0.582	-0.172	-0.124	-0.232	-0.331	-0.269
Hydropsychidae	-0.437	0.369	-0.555	-0.127	-0.016	0.500	0.232	0.160
Leptophlebiidae	0.281	-0.120	0.388	0.209	0.171	0.225	0.214	0.305
Libellulidae	0.241	-0.140	0.299	0.260	0.216	0.319	0.257	0.402
Lumbriculidae	-0.603	0.598	-0.026	0.769*	-0.889**	-0.727	-0.782*	-0.664
Lymnaeidae	0.191	-0.003	0.351	0.093	0.239	0.393	0.490	0.066
Naididae	0.188	0.123	-0.015	-0.093	0.022	-0.098	0.004	-0.254
Planorbidae	0.811*	-0.928**	0.393	0.817*	0.659	0.017	0.180	0.587
Protoneuridae	0.289	-0.353	0.662	0.408	0.296	0.389	0.412	0.613
Thiaridae	0.057	0.160	-0.496	0.023	0.198	0.287	0.167	-0.055
Tubificidae	0.304	-0.378	-0.085	0.302	0.328	-0.029	0.084	0.044
Unidentified Oligochaeta	0.162	-0.269	-0.491	0.401	0.407	0.300	0.167	0.414
Viviparidae	-0.213	-0.129	-0.160	-0.364	-0.533	-0.802*	-0.811*	0.269

Note: * Correlation is significant at $p<0.05$; ** Correlation is significant at $p<0.01$; WT = Water Temperature the low dissolved oxygen present at sampling sites near to the fish farm effluent points (SR1, SR3, SR4, and SR5). Generally, a high concentration of BOD and COD indicates organic pollution in urban rivers and *vice versa*. Similar to the current study, Popovic *et al*. (2016) disclosed a positive relationship of the above water quality parameters and Chironomidae, and has identified this family as a pollution-tolerant taxa in Kolubara River, Serbia, which is impacted by various anthropogenic activities. Also, Frizzera and Alves (2012) discovered that the abundance of many aquatic Oligochaeta has a positive relationship with BOD, COD, and nutrients in an urban stream in Brazil.

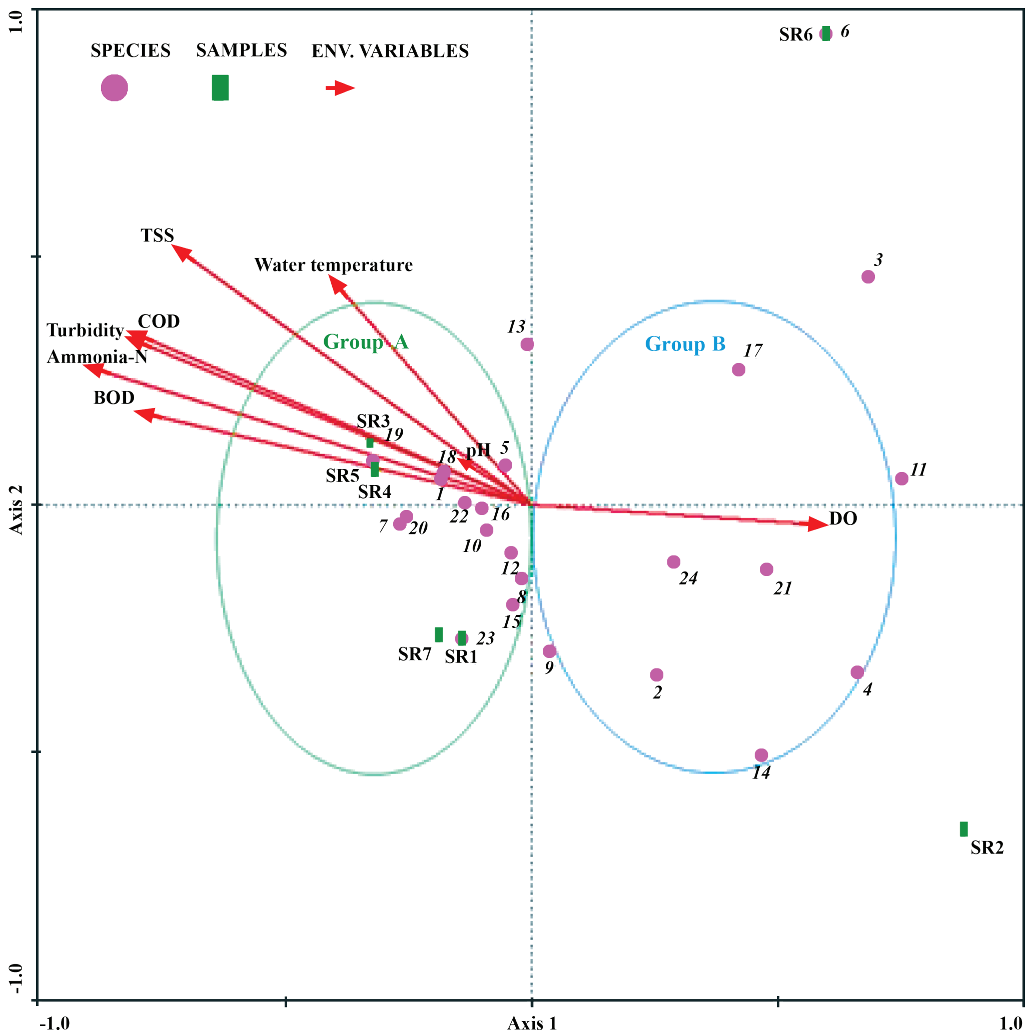

Figure 3. Canonical Correspondence Analysis triplot showing the abundance distribution of benthic macroinvertebrates by water quality parameters; 1 = Aeolosomatidae; 2 = Atyidae; 3 = Baetidae; 4 = Caenidae; 5 = Chironomidae; 6 = Corbiculidae; 7 = Coenagrionidae; 8 = Corduliidae; 9 = Erpobdellidae; 10 = Glossiphoniidae; 11 = Gomphidae; 12 = Haplotoxidae; 13 = Hydropsychidae; 14 = Leptophlebiidae; 15 = Libellulidae; 16 = Lumbriculidae; 17 = Lymnaeidae; 18 = Naididae; 19 = Planorbidae; 20 = Protoneuridae; 21 = Thiaridae; 22 = Tubificidae; 23 = Unidentified Oligochaeta; 24 = Viviparidae; Longer arrows denote a more decisive impact on the benthic macroinvertebrates and sampling sites and *vice versa*.

The high abundance of some families (Figure 3) noticeably distinguished Group B. The families Caenidae, Gomphidae, Aytidae, Leptophlebiidae, Thiaridae, and Viviparidae (considered highly and moderately sensitive) were positively related to high DO at the sampling site with no disturbance from fish farm wastes (reference site/SR2). They were negatively related to low DO, as sampling sites disturbed from high organic matter in fish farm discharges supported high BOD_5, COD, TSS, NH_3, and turbidity. These factors were more toxic to benthic macroinvertebrates at low pH. These families were found at sites with high/moderate DO concentrations and in high abundance in areas less affected by fish farms.

Some researchers found a positive correlation between DO concentration and the family Aytidae in Periurban stream, Cameroon (Samuel *et al*., 2012), family Leptophlebiidae in spring-fed headwaters of the Doon Valley, India (Mishra *et al*., 2020), and family Caenidae in streams of Gunung Tebu Forest Reserve, Terengganu (Md Rawi *et al*., 2014). Hence, Order Epheomeptera is a pollution-sensitive benthic macroinvertebrate group, and they may not tolerate low DO concentrations.

Similar to the current study, a survey conducted in Aiba Stream, Iwo, southwestern Nigeria by Akindele and Liadi (2013) showed that Valvatidae was highly dependent on DO, whereas Edegbene (2020) found that Thiaridae was moderately dependent on DO at a dam in northwestern Nigeria. Hence, DO concentration is a crucial parameter affecting the distribution of aquatic organisms. According to the observed correlation between the relative abundance of each family and water quality parameters, similarities can be drawn with polluted urban rivers. However, correlations between the abundance of benthic macroinvertebrates and water quality would likely differ in the recreational or upstream areas of the rivers.

Morphological adaptation of benthic macroinvertebrates to survive under influence of organic pollution

The CCA also revealed groups of macroinvertebrates that are highly and moderately tolerant, and sensitive to organic pollution resulting from fish farming, similar to the previous studies (Akindele and Liadi, 2013; Pyron and Brown, 2015). Planorbidae were found to be a pollution-tolerant taxon in the current study. They have no primary gills for oxygen intake and are adaptable to low dissolved oxygen because they are able to use a conical extension of the epithelium as a gill. They also have hemoglobin, a respiratory pigment (Pyron and Brown, 2015). These biological characteristics also improve oxygen transport efficiency under the organically polluted condition of rivers.

Several previous studies (e.g., Othman *et al*., 2012; Namin *et al*., 2013) and the current study have demonstrated that family Naididae is a good indicator of organic pollution. Therefore, Naididae could be considered a pollution-tolerant taxon because individuals can tolerate poor water quality by using external gills located either at the forebody, hind body, or surrounding the posterior end (Timm, 2012). The body's posterior part takes in oxygen via the body wall, but the anterior part is normally submerged in the substratum. As a result, the oxygen concentration in the anterior body part is reduced. Gaseous exchange is intensified through rhythmic movements of the tail, and oxygen is spread throughout the entire body through the movement of the gut muscles (Haaren and Soors, 2013). Some members of Naididae also have an additional respiratory system to survive in low oxygen conditions. Due to these biological characteristics, Naididae can tolerate organically polluted water.

Similar to previous studies (Vivien *et al*., 2015), the present study identified Lumbriculidae as a pollution-tolerant taxon. They have a well-developed blood circulatory vascular system with branched and blinded lateral vessels, presumably to improve respiration (Timm and Martin, 2015). Some Lumbriculids are sediment dwellers that feed on small organic matter and obtain their nutrition from bacteria that live on the organic material. When the organic load becomes too high, many Oligochaeta species will die because of the lack of food and oxygen near the sediment. Only a few species can survive these conditions. Those that can have good blood circulation and a dense vascular system, like some Lumbriculidae. Therefore, this morphological adaptation assists some Lumbriculidae in breathing in aquatic environments with low dissolved oxygen.

Similar to Martins *et al*. (2008) and Vivien *et al*. (2015), the current study identified Tubificidae as a pollution-tolerant taxon. Some Tubificidae species have respiratory pigments (Glasby *et al*., 2021) to improve their oxygen-exchange efficiency. Hence, they can tolerate low oxygen concentrations. Usually, gas is exchanged through the thin body wall in aquatic oligochaetes. However, like some Naididae, Tubificidae pumps water into the anus to increase gas exchange available in the body wall surface area. Therefore, those taxa can survive and live in habitats with low dissolved oxygen due to these additional respiratory mechanisms.

Chironomidae is also identified as a pollution-tolerant taxon because its members can tolerate extreme hypoxic conditions in aquatic habitats as they have high hemoglobin levels within their bodies (Nath, 2018), which play a vital physiological role in increasing respiratory efficiency. Besides, most chironomid larvae living in low-oxygen sediments construct burrows and fixed tubes of sediment held together with silky secretions. Tube- and burrow-dwellers can ventilate their tubes with fresh water by dorsoventral undulations of the body, facilitating gas exchange during low ambient oxygen. Therefore, Chironomidae has been reported as the dominant order in the most organically contaminated ecosystems.

The current study noted that Ephemeroptera (i.e., Leptophlebiidae and Caenidae) is a pollution-sensitive taxon. They cannot survive in high TSS and turbidity due to the associated low dissolved oxygen concentration. Ephemeroptera have an external respiratory surface as a morphology characteristic. These gills can be easily clogged with suspended solids due to colloid particles in the water. The gills cannot function properly and extract oxygen from the water when clogged, and this could be lethal to ephemeropterans. Another of the dominant taxa identified, Aytidae has a similar mechanism of gas exchange through the diffusion process across the body surface due to gills. Usually, gills are convoluted outgrowths containing blood vessels covered by a thin epithelial layer (Thorp and Lovell, 2014). Therefore, the presence of gills highly increases the gas exchange surface area.

According to the observed moderately pollution-sensitive taxa, Thiaridae and some Viviparidae have primary internal gills through which surrounding water can easily access their bodies. This biological structure consists of a series of narrow flat leaflets well supplied with blood and arranged like the teeth of a comb. Therefore, these gill snails can obtain a small amount of oxygen through the general body surface.

CONCLUSION

The present study showed that DO, ammonia-nitrogen, TSS, BOD_5, and COD could be indicators of organic pollution produced by fish farming activities and thus impacted the Rawang sub-basin of the Selangor River. Furthermore, a correlation was noted between benthic macroinvertebrate communities and water quality in the sites disturbed by fish farming activities. Families Aeolosomatidae, Chironomidae, Lumbriculidae, Naididae, Planorbidae, and Tubificidae were organic pollution tolerant, whereas Caenidae, Gomphidae, Aytidae, Leptophlebiidae, Thiaridae, and Viviparidae were highly or moderately sensitive to organic pollution. It is noteworthy that all these organic pollution-tolerant benthic macroinvertebrates dominated the fish farm sites and thus, indicated that these macroinvertebrates are good indicators for detecting organic pollution and poor water quality in the stream habitat influenced by the fish farms' effluents.

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