UNIVERSITI PUTRA MALAYSIA

APPLICATION OF MULTIMETRIC MODEL INVOLVING MACROBENTHOS BIOINDICATORS IN ASSESSING ORGANIC CONTAMINATION IN RAWANG SUB-BASIN, SELANGOR RIVER, MALAYSIA

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

This Ph.D. thesis is incredibly dedicated to the following most patient persons in my life who made the impossible possible.

My parents and siblings

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

APPLICATION OF MULTIMETRIC MODEL INVOLVING MACROBENTHOS BIOINDICATORS IN ASSESSING ORGANIC CONTAMINATION IN RAWANG SUB-BASIN, SELANGOR RIVER, MALAYSIA.

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Organic pollution due to unsustainable fish farming activities is a common problem in the Rawang sub-basin of the Selangor River. However, no comprehensive study has evaluated the organic contamination potential caused by fish farm effluents using local macrobenthos communities. The objectives of this study were: i) to assess macrobenthos assemblages, habitat quality, and water quality within the fish farming areas, ii) to establish potential macrobenthos as bioindicators for organic pollution assessment, and iii) to improvise a multimetric model using macrobenthos for organic pollution assessment. This study chose six sampling sites based on accessibility and proximity to fish farms, with one sampling site as a reference site. Macrobenthos and water sampling were completed from April 2019 to March 2020, and physical habitats were assessed during rainy and dry seasons. The macrobenthos assemblages, habitat quality, and water quality were evaluated using different indices. Principal Components Analysis (PCA) and Canonical Correspondence Analysis (CCA) helped determine the potential of organic pollution indicators. The reference site was excluded when verifying the bioindicators and the model improvised due to the significant differences in water quality parameters and the macrobenthos composition. Suitable bioindicators for applying a model for organic contamination assessment were determined using PCA. The backward multiple linear regression (MLR) was employed to determine the significant difference of identified bioindicators with water quality parameters. Based on the score value (PCA variance coefficient) of each macrobenthos family, the cumulative score value of each sampling site was calculated by considering each replicate as a sampling site to increase the number of samples (i.e., 18 = sampling sites 6 x 3 replicates). The cumulative score values of the sampling sites were classified using cluster analysis. The resultant dendrogram produced three clusters. The cluster range value and mean confidence intervals were used to obtain a distinct classification of water quality classes. The improvised model of water quality standards was validated internally and externally. Results revealed that organic effluent originating from fish farming practices affected river health. The unsustainable fish farming activities mainly influenced the organic contamination water quality parameters (EC, DO, BOD, COD, pH, and ammoniacal-nitrogen) and macrobenthos bioindicators. The CCA showed many pollution-tolerant and moderately pollution-tolerant taxa (Aeolosomatidae, Chironomidae, Lumbriculidae, Naididae, Planorbidae, and Tubificidae) were affected by the high BOD, COD, turbidity, TSS, EC, and ammoniacal-nitrogen. The families Gomphidae, Aytidae, Leptophlebiidae, Thiaridae, and Viviparidae were sensitive to pollution and affected by DO concentration. Based on the multivariate statistical analysis, nine macrobenthos families (Baetidae, Libellulidae, Protoneuridae, Chironomidae, Corbiculidae Hydropchysidae, Tubificidae, Lumbriculiade, and Naididae) were identified as bioindicators to improvise the model. Based on the mean confidence intervals for each cluster range, three different value scales were developed to represent the contamination level (i.e., <0.69 as organically polluted, 0.69 - 0.87 as slightly organic polluted, and >0.87 as clean status). The results produced after validation were better than the water quality status from other studies based on the BMWP/BMWP^{Thai} score. This study concludes that an improvised multimetric model can evaluate river organic contamination successfully.

Keywords: Freshwater quality, fish farming, organic pollution, and multimetric model

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

APLIKASI MODEL MULTIMETRIK YANG MELIBATKAN BIOINDICATOR MACROBENTHOS DALAM MENILAI PENCEMARAN ORGANIK DI SUB-LEMBANGAN RAWANG, SUNGAI SELANGOR, MALAYSIA.

Oleh

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Pencemaran organik akibat aktiviti penternakan ikan yang tidak mampan merupakan masalah biasa di sub-lembangan Sungai Selangor di Rawang. Walau bagaimanapun, tiada kajian menyeluruh untuk menilai potensi pencemaran organik yang disebabkan oleh efluen daripada kolam ikan dengan menggunakan komuniti makrobenthos tempatan. Objektif kajian ini adalah: i) untuk menilai kumpulan makrobentos, kualiti habitat, dan kualiti air dalam kawasan penternakan ikan, ii) untuk mewujudkan potensi makrobentos sebagai bioindikator untuk penilaian pencemaran organik, dan iii) untuk menambah baik model multimetrik menggunakan macrobenthos bagi penilaian pencemaran organik. Enam tapak persampelan berdasarkan ketersampaian kawasan dan berdekatan dengan kolam ikan yang dipilih untuk kajian ini, dan satu tapak persampelan sebagai tapak rujukan. Pensampelan makrobentos dan air telah dilaksanakan pada April 2019 hingga Mac 2020, dan habitat fizikal dinilai semasa musim hujan dan musim kering. Kumpulan makrobenthos, kualiti habitat, dan kualiti air dinilai menggunakan indeks yang berbeza. Analisis Komponen Utama (PCA) dan Analisis Koresponden Kanonik (CCA) membantu menentukan potensi penunjuk pencemaran organik. Tapak rujukan telah dikecualikan ketika mengesahkan bioindikator dan model yang diubahsuai kerana perbezaan ketara dalam parameter kualiti air dan komposisi makrobenthos. Bioindikator yang sesuai untuk menggunakan model bagi penilaian pencemaran organik ditentukan menggunakan PCA. Regresi linear berganda ke belakang (MLR) digunakan untuk menentukan perbezaan ketara bioindikator yang dikenal pasti dengan parameter kualiti air. Setiap famili makrobenthos, nilai skor kumulatif setiap tapak persampelan dikira berdasarkan nilai skor (pekali varians PCA), dengan mempertimbangkan setiap replika sebagai tapak persampelan untuk meningkatkan bilangan sampel (iaitu, 18 = tapak persampelan 6 x 3 ulangan). Nilai skor kumulatif tapak persampelan dikelaskan menggunakan analisis kelompok. Dendrogram menghasilkan tiga kelompok. Nilai julat kelompok dan selang keyakinan min digunakan untuk mendapatkan klasifikasi kelas kualiti air yang berbeza. Model piawaian kualiti air yang telah diubahsuai telah disahkan secara dalaman dan luaran. Keputusan menunjukkan bahawa efluen organik yang berasal daripada amalan penternakan ikan menjejaskan kesihatan sungai. Aktiviti penternakan ikan yang tidak mampan mempengaruhi parameter kualiti air pencemaran organik (EC, DO, BOD, COD, pH, dan ammoniakal-nitrogen) dan bioindikator makrobenthos. CCA menunjukkan banyak takson toleran pencemaran dan sederhana toleran pencemaran (Aeolosomatidae, Chironomidae, Lumbriculidae, Naididae, Planorbidae, dan Tubificidae) telah terjejas oleh BOD, COD, kekeruhan, TSS, EC dan ammoniacal-nitrogen yang berkepekatan tinggi. Famili Gomphidae, Aytidae, Leptophlebiidae, Thiaridae, dan Viviparidae adalah sensitif terhadap pencemaran, dan dipengaruhi oleh kepekatan DO. Berdasarkan analisis statistik multivariat, Sembilan famili makrobenthos (Baetidae, Libellulidae, Protoneuridae, Chironomidae, Corbiculidae Hydropchysidae, Tubificidae, Lumbriculiade, dan Naididae) dikenal pasti sebagai bioindikator dalam model yang ditambah baik. Berdasarkan selang keyakinan min bagi setiap julat kelompok, tiga skala nilai yang berbeza telah dibangunkan untuk mewakili tahap pencemaran (iaitu, <0.69 sebagai tercemar secara organik, 0.69 - 0.87 sebagai sedikit tercemar organik, dan >0.87 sebagai status bersih). Keputusan yang dihasilkan selepas pengesahan adalah lebih baik daripada status kualiti air daripada kajian lain berdasarkan skor BMWP/BMWP^{Thai}. Kajian ini menyimpulkan bahawa improvasi model multimetrik daripada kajian ini-boleh menilai pencemaran organik sungai dengan jayanya.

Kata kunci: Kualiti air tawar, penternakan ikan, pencemaran organik, dan model multimetrik

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ASPT	Average Score per Taxon
BOD	Biochemical Oxygen Demand
BMWP	Biological Monitoring Working Party
CCA	Canonical Correspondence Analysis
COD	Chemical Oxygen Demand
DOE	Department of Environment
DOF	Department of Fisheries
DID	Department of Irrigation and Drainage
D_{Mg}	Margalef Diversity Index
DO	Dissolved Oxygen
EC	Electrical Conductivity
eDNA	Environmental DNA
EPT	Ephemeroptera, Plecoptera, Trichoptera
FBI	Family Biotic Index
GIS	Geographic Information System
IDW	Inverse Distance Weighted
KMO	Kaiser-Meyer-Olkin
MLR	Multiple Linear Regression
NWQS	National Water Quality Standards
PCA	Principal Component Analysis
QHEI	Qualitative Habitat Evaluation Index
H’	Shannon’s Diversity Index
D	Simpson’s Diversity Index
SS	Suspended Solids
TDS	Total Dissolved Solids

TSS	Total Suspended Solids
UN	United Nations
USDA	United States Department of Agriculture
WQI	Water Quality Index
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

The river system is important as a natural entity that provides critical water resources for numerous ecosystem functions. It is used for various purposes in daily life, such as domestic consumption, industrial purposes, hydroelectricity generation, recreation, tourism, fishing, and agricultural activities. However, these anthropogenic activities increase river water pollution (Liyanage and Yamada, 2017). Among the water pollution types, organic pollution occurs when large quantities of organic compounds in wastewater are released from various anthropogenic activities (Wen et al., 2017). Therefore, monitoring the health of streams and rivers is vital.

A river health assessment is currently conducted mainly based on the assessment of water quality, biota, and physical habitat. The river is a complex ecosystem; hence using a single factor cannot clearly picture the river's ecological health (Sabater and Elosegi, 2014; Wei et al., 2009). Traditionally, physicochemical parameters assess the quality of river water. However, determining physicochemical parameters is commonly cost-intensive, time-consuming, and dependent on particular instruments used. Similarly, physicochemical parameters can fluctuate over time and only show environmental conditions at the time of measurement (Aazami et al., 2015).

Aquatic organisms are currently recognized as excellent bioindicators for many reasons. They can assist in evaluating the long-term environmental conditions and classify the river's ecological status (Azmi et al., 2018; Ojija and Laizer, 2016). The most frequently used methods are evaluating stream health using aquatic organisms such as fish, periphyton, macrobenthos, and microbes. Among these, macrobenthos are the most commonly used and have the most extended history of use in biomonitoring (Azmi et al., 2018; Jun et al., 2012) due to their high biodiversity, relatively long life-span, bottom-dwelling lifestyle, and sensitivity towards environmental changes (Selvanayagam and Abril, 2016). Therefore, the macrobenthos are good biological indicators to determine the anthropogenic impacts on freshwater ecosystems.

As a result, the escalating deterioration in the quality of freshwater resources implies the development stipulation of obligatory tools, methodology, and approaches in quantifying the impact of human-induced activities on freshwater ecosystems (Edegbene et al., 2019). Based on the previously developed models, macrobenthos are the essential tools for assessing biological resources' quality (Mehrjo et al., 2020). After the saprobic system in 1902, Europe begun to use biomonitoring methods for river quality monitoring (Capo-Chichi et al., 2021). Afterward, some researchers in tropical countries have attempted to develop macrobenthos-based models for river quality monitoring (Musonge et al., 2020; Mustow, 2002) because local macrobenthos alter from one ecological region to other ecological regions (Musonge et al., 2020; Blakely et al., 2014).

1.2 Problem Statement

Organic pollution is a common river water pollution type in tropical rivers. Fish farming is one of the main activities contributing to organic pollution. Most fish farms in Malaysia use river water as their primary water source. The Selangor River is one example. The environmental impacts from fish farming activities arise due to the release of excess nutrients and antibiotics to the surrounding environment and the introduction of invading species (Kawasaki et al., 2016b). Among the several anthropogenic activities, fish farming facilitates organic pollution.

Organic pollution occurs when excess organic matter such as manure and sewage enters the river water (Wen et al., 2017). Dissolved and suspended solids (SS), Biochemical Oxygen Demand (BOD), ammonia, and nutrients such as phosphate and nitrate are vital indicators of organic pollution. The release of untreated effluent from unsustainable fish farming practices into nearby rivers may decrease biodiversity and create environmental and ecological impacts (Aubin et al., 2019). Previous studies have focused on the effects of organic pollution arising from fish farming practices on the river water quality of the Selangor River in Selangor (Kawasaki et al., 2016a). However, no studies have yet investigated the influence of organic pollution on macrobenthos communities in Malaysia's streams and rivers.

Organic pollution can be determined by assessing and integrating water quality parameters and macrobenthos. Many models have been developed in Malaysia to assess river water quality using physicochemical parameters (Chowdhury et al., 2018). However, a model must be improvised to assess organic pollution using macrobenthos as bioindicators in Malaysian rivers. Hence, improvising a model based on organic contamination is a significant positive step towards effectively determining the organic pollution of rivers in the future.

1.3 Significance of the study

Assessment of river health is challenging due to the diversity and functional service of the river. Though river organic pollution is a common scenario, there is no well-improvised model for assessing river organic contamination using macrobenthos as bioindicators in Malaysia. Therefore, such results are essential for the effective management and restoration of river ecosystems, especially for Malaysian rivers in the future.

The outcome of this study is also expected to use by various government agencies, the Department of Fisheries (DOF), the local authority in Selangor, the Department of Environment (DOE), and other relevant stakeholders regarding the prevention of river pollution. The public at large will also benefit from the application of bioindicators by concerned authorities. Furthermore, this study provides the baseline information of the Selangor River's macrobenthos as bioindicators by filling the knowledge gaps of the distribution of macrobenthos in Malaysian rivers.

1.4 Research questions

1. How does organic contamination affect the health of the receiving water?
2. How do fish farming practices influence the macrobenthos assemblages within the ecosystem?
3. How can macrobenthos be used to determine organic contamination?
4. Can the newly improved multimetric model be used to assess the level of organic contamination of the river?

1.5 Objectives

- To assess macrobenthos assemblages, habitat quality, and water quality within fish farming areas.
- To reveal potential macrobenthos as bioindicators for organic pollution assessment.
- To improvise a multimetric model using macrobenthos for organic contamination assessment.

1.6 Hypotheses

1. H_o: Unsustainable fish farming does not produce any significant water quality change.

   H_1: Unsustainable fish farming produces any significant water quality change.
2. H_o: Macrobenthos do not have significant potential as biological indicators for organic pollution assessments.

   H_1: Macrobenthos have significant potential as biological indicators for organic pollution assessments.

1.7 Scope of the study

This study aims to improvise the model using macrobenthos as bioindicators to assess organic contamination due to fish farming activities. Hence, this newly improvised model is only suitable for organic contamination determination. It must be modified relevant to the particular area's local condition if used by other countries. This survey also covers river health assessment using macrobenthos assemblages, physical habitat, and water quality.

The Selangor River is a 110 km long massive river covering approximately 2200 m^2 of the catchment area. Therefore, this study tested only one sub-basin of the Selangor River. The Rawang sub-basin is one of the vital fish farming areas in the Selangor River. However, each river in the sub-basin did not have a fish farm. Accessibility to all fish farms near the river is impossible. Thus, the study neither selected all sites in proximity to fish farm sites nor considered all sub-basin rivers of the Selangor River.

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APPENDICES

Appendix A

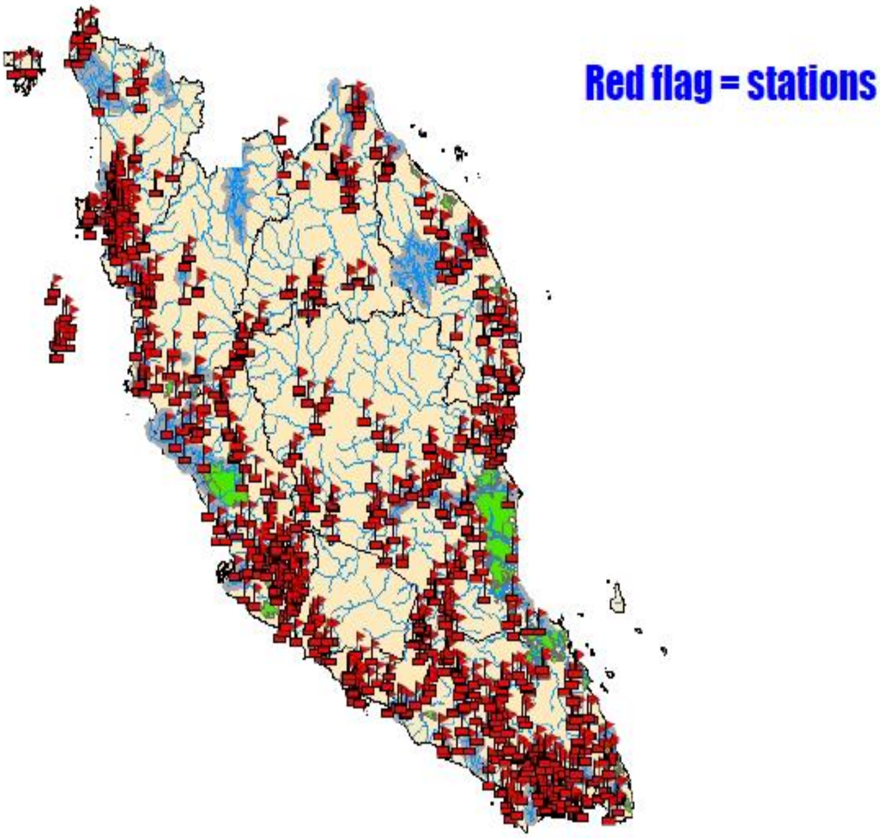


Figure A1 : Distribution of DOE water quality monitoring stations in Peninsular Malaysia
(Source:DOE, Malaysia: Accesible at http://www.wepa-db.net/pdf/0810malaysia/f.pdf)

Appendix B

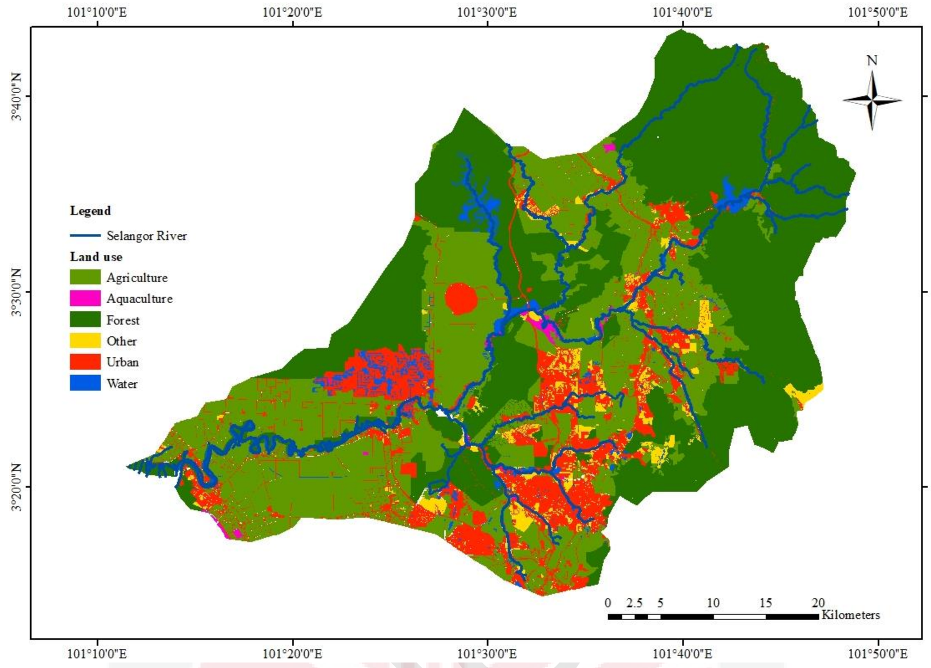


Figure B1 : Land use map of the Selangor River basin in 2017

Appendix C

SR 1 : Guntong River

SR 2 : Guntong River tributary

SR 3 : Kuang River

SR 4 : Gong River

SR 5 : Buaya River

SR 6 : Serendah River

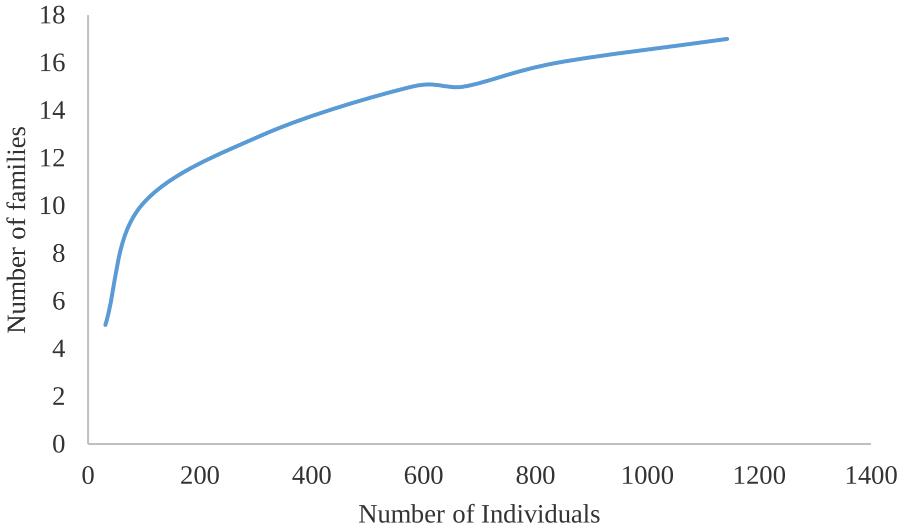
SR 7 : Kuang River

Figure C1 : Views of the sampling sites

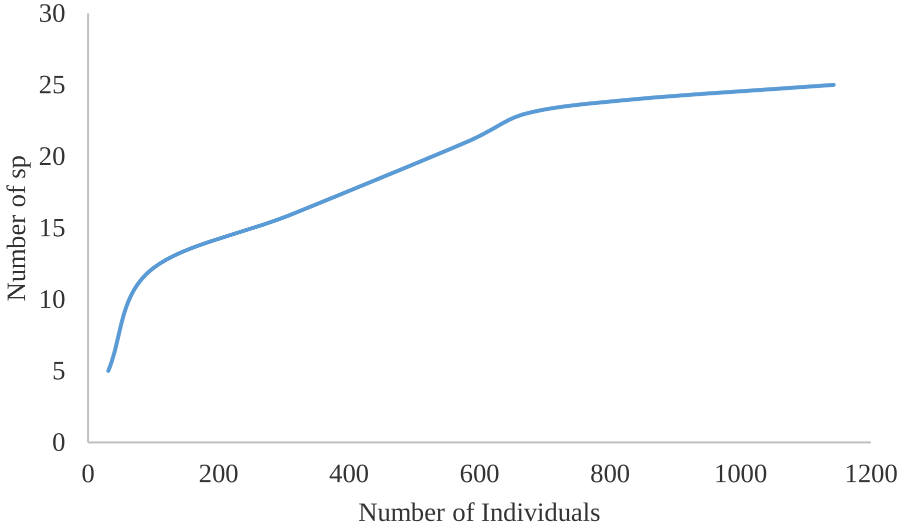
Appendix D

SR 1

Family level



Genus level



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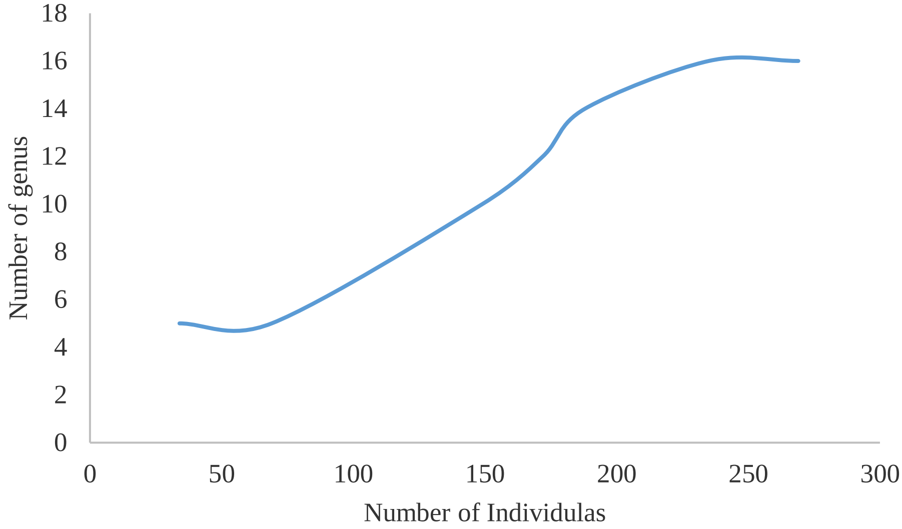
Family level



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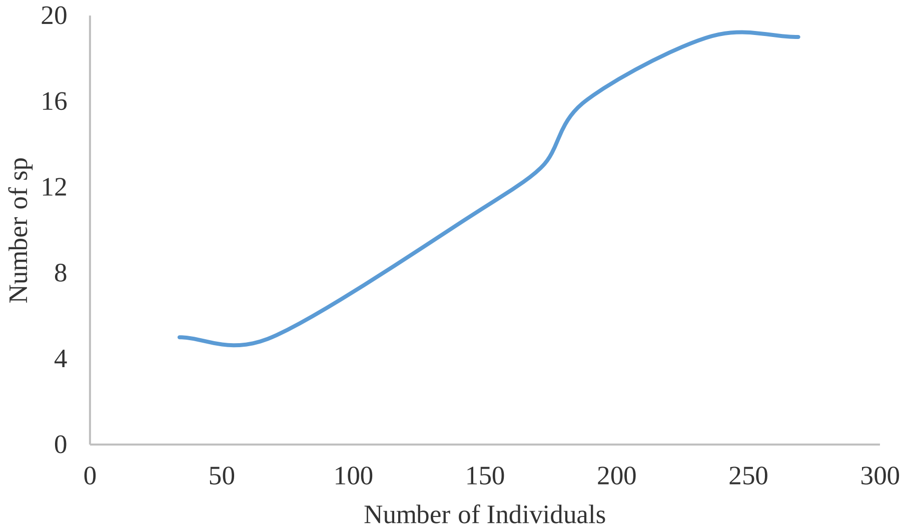
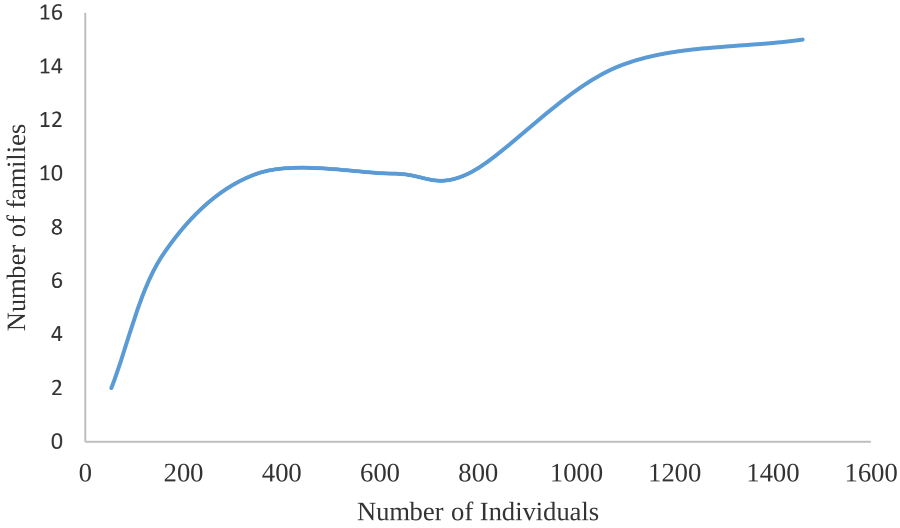


SR 3

Family level



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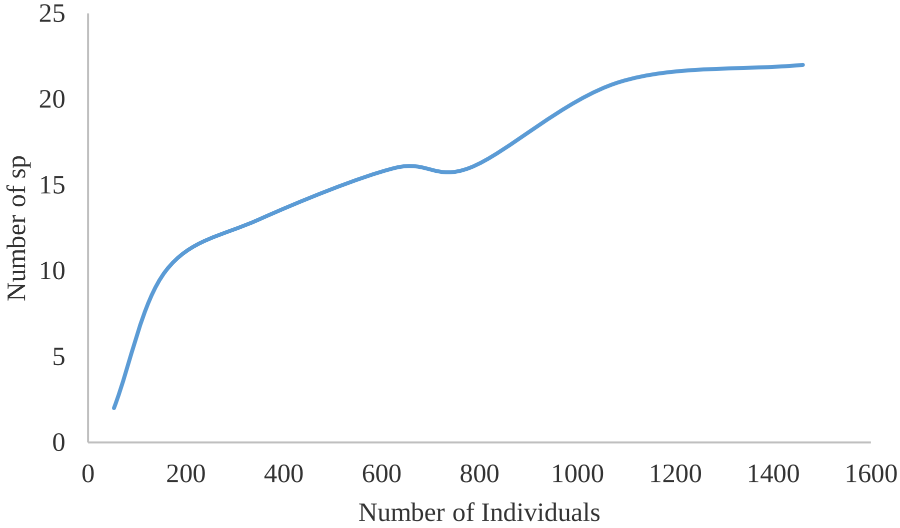

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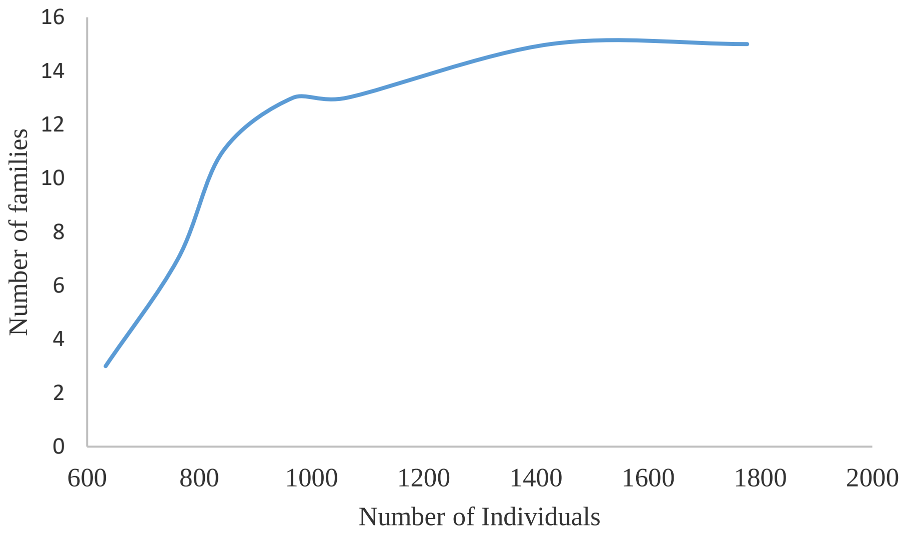
Family level



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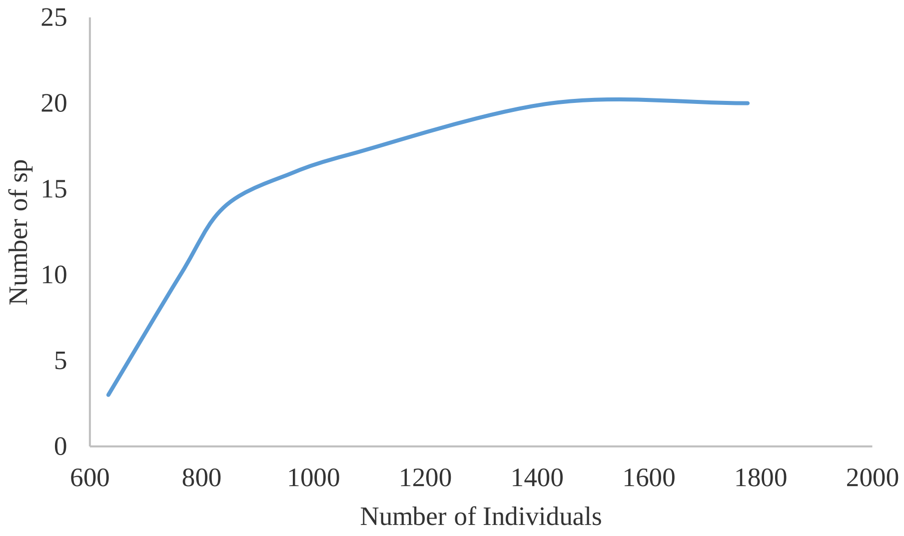
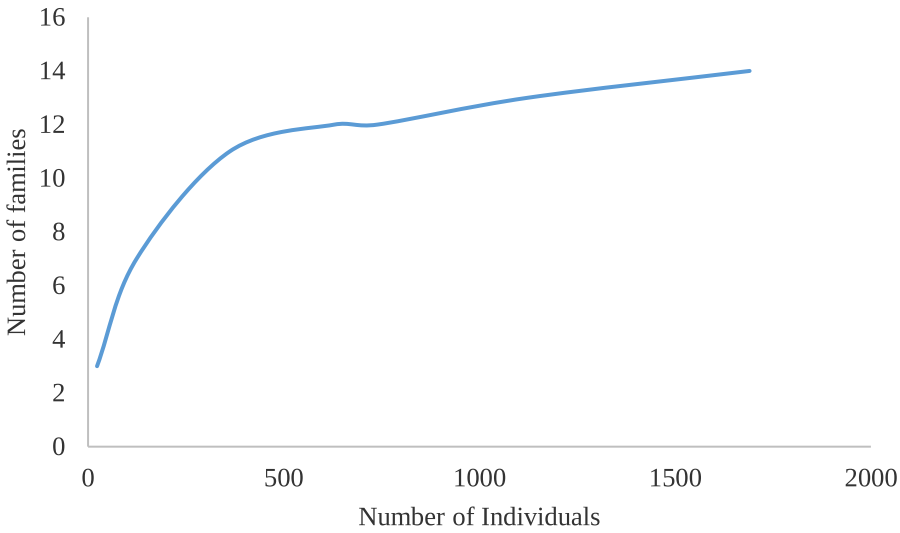


SR 5

Family level



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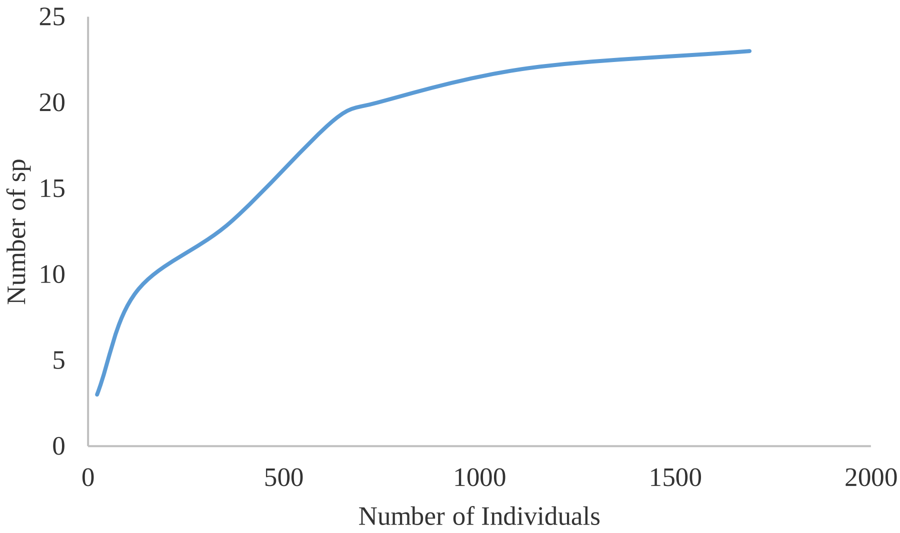
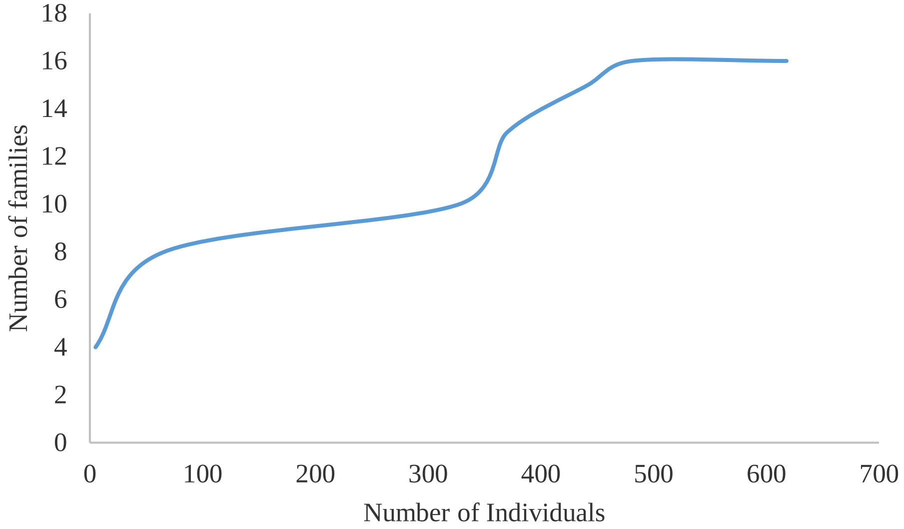
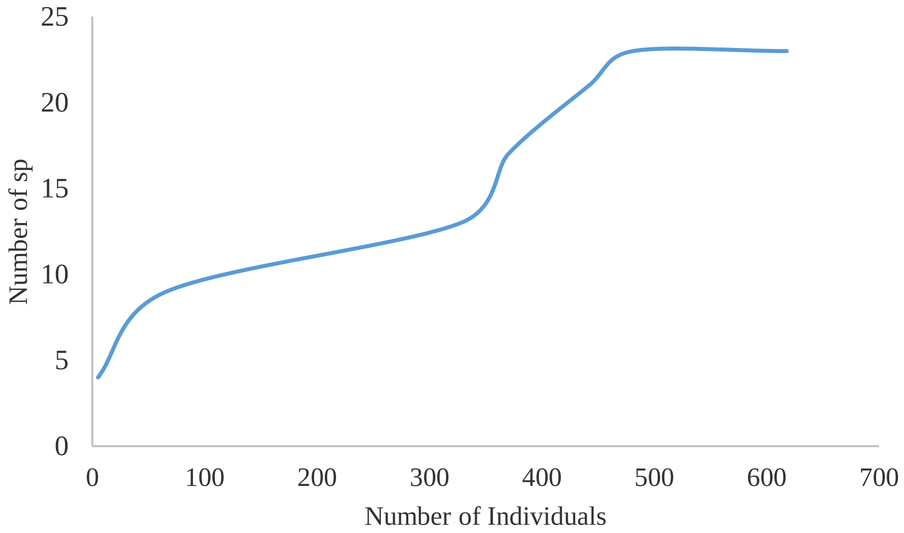


SR 6

Family level

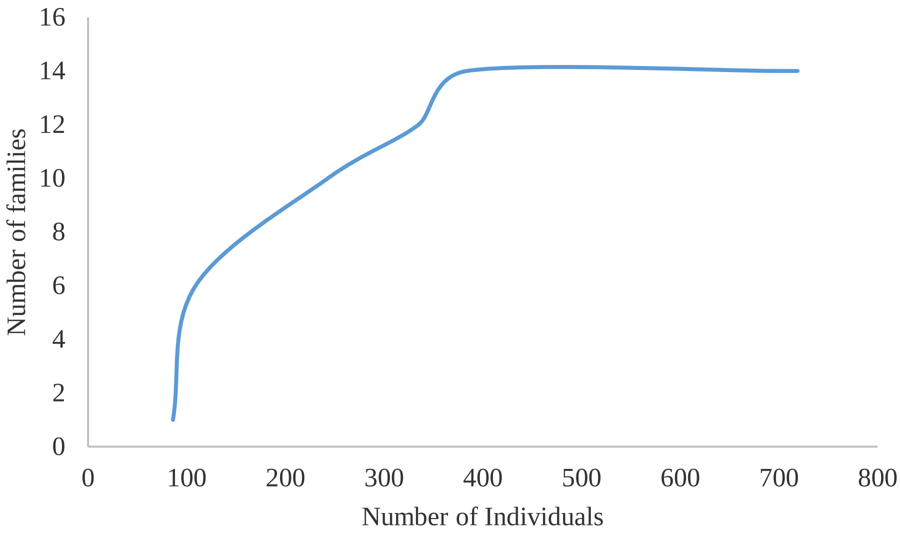


Genus level



SR 7

Family level



Genus level

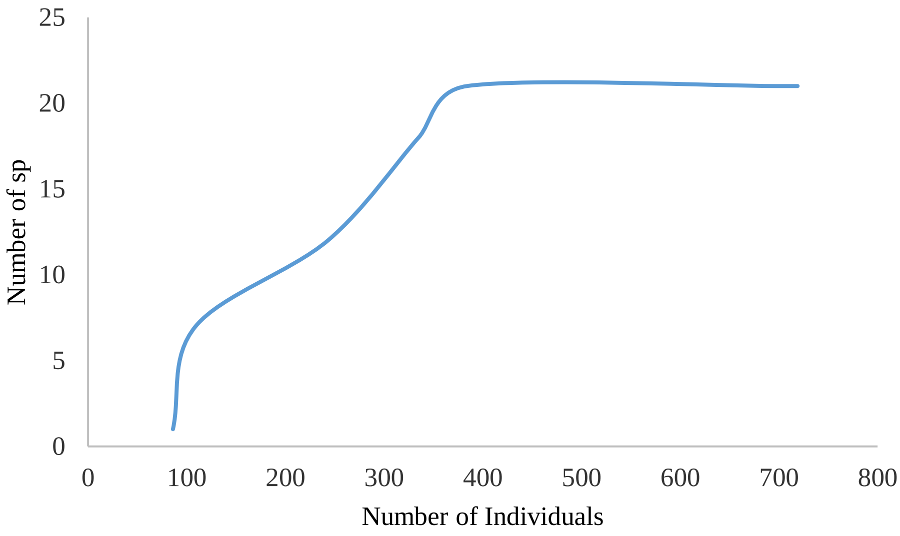


Figure D1 : Rarefaction curves based on the family and the genus levels in each sampling sites

Appendix E

Table E1 : Species level pollution tolerances score for FBI

Family	**Genus**	**Tolerance value**
Aeolosomatidae	*Aeolosoma* sp.	8
Atyidae	-	6
Baetidae	*Procloeon* sp.	4
Caenidae	*Caenoculis* sp.	7
Chironomidae	*Chironomus* sp.	10
Chironomidae	*Cryptochironomus* sp.	8
Chironomidae	*Polypedilum* sp.	6
Chironomidae	*Rheotanytarsus* sp.	6
Chironomidae	Tanypodinae	6
Cladocera	-	
Corbiculidae	*Batissa* sp.	6
Corbiculidae	*Corbicula* sp.	6
Coenagrionidae	Agriocnemis sp.	9
Corduliidae	*Cordulia* sp.	5
Dytiscidae	*Dytiscus* sp.	
Ephydridae	*Brachydeutera* sp.	
Erpobdellidae	*Erpobdella* sp.	8
Glossiphoniidae	*Helobdella* sp.	8
Gomphidae	*Hagenius* sp.	3
Gomphidae	*Paragomphus* sp.	
Haplotaxidae	Haplotaxidae	5
Hydropsychidae	*Amphipsyche* sp.	4
Leptophlebiidae	*Thraulus* sp.	2
Libellulidae	*Brachythemis* sp.	9
Libellulidae	*Libellula* sp.	8
Libellulidae	*Neurothemis fluctuans*	9
Libellulidae	*Orthetrum* sp.	9
Libellulidae	*Sympetrum* sp.	7
Lumbriculidae	-	5
Lymnaeidae	*Lymnaea* sp.	6
Naididae	*Aulophorus* sp.	8
Naididae	*Branchiodrilus hortensis*	8
Naididae	*Dero* sp.	10
Naididae	*Nais* sp.	8
Naididae	*Pristina* sp.	8
Planorbidae	Planorbidae	7
Protoneuridae	*Prodasineura* sp.	-
Thiaridae	*Melanoides* sp.	6
Thiaridae	*Thiara* sp.	6
Tubificidae	*Branchiura sowerbyi*	6
Tubificidae	Tubificidae	10
Unidentified Oligochaeta	-	8
Viviparidae	*Filopaludina* sp.	6
Viviparidae	Viviparidae	6

Appendix F

Table F1 : Family level pollution tolerances score for BMWP^{Thai}

Family	Genus	Tolerance value
Aeolosomatidae	*Aeolosoma* sp.	1
Atyidae	-	8
Baetidae	*Procloeon* sp.	4
Caenidae	*Caenoculis* sp.	7
Chironomidae	*Chironomus* sp.	2
Chironomidae	*Cryptochironomus* sp.	2
Chironomidae	*Polypedilum* sp.	2
Chironomidae	*Rheotanytarsus* sp.	2
Chironomidae	Tanypodinae	2
Cladocera	-	-
Corbiculidae	*Batissa* sp.	3
Corbiculidae	*Corbicula* sp.	3
Coenagrionidae	*Agriocnemis* sp.	6
Corduliidae	*Cordulia* sp.	6
Dytiscidae	*Dytiscus* sp.	5
Ephydridae	*Brachydeutera* sp.	-
Erpobdellidae	*Erpobdella* sp.	3
Glossiphoniidae	*Helobdella* sp.	3
Gomphidae	*Hagenius* sp.	6
Gomphidae	*Paragomphus* sp.	6
Haplotaxidae	Haplotaxidae	1
Hydropsychidae	*Amphipsyche* sp.	5
Leptophlebiidae	*Thraulus* sp.	10
Libellulidae	*Brachythemis* sp.	6
Libellulidae	*Libellula* sp.	6
Libellulidae	*Neurothemis* sp.	6
Libellulidae	*Orthetrum* sp.	6
Libellulidae	*Sympetrum* sp.	6
Lumbriculidae	-	1
Lymnaeidae	*Lymnaea* sp.	3
Naididae	*Aulophorus* sp.	1
Naididae	*Branchiodrilus hortensis*	1
Naididae	*Dero* sp.	1
Naididae	*Nais* sp.	1
Naididae	*Pristina* sp.	1
Planorbidae	Planorbidae	3
Protoneuridae	*Prodasineura* sp.	3
Thiaridae	*Melanoides* sp.	3
Thiaridae	*Thiara* sp.	3
Tubificidae	*Branchiura sowerbyi*	1
Tubificidae	Tubificidae	1
Unidentified Oligochaeta	-	1
Viviparidae	*Filopaludina* sp.	6
Viviparidae	Viviparidae	6

Appendix G

Table G1 : Habitat Assessment Field Data Sheet—High Gradient Streams (Front)

STREAM NAME	LOCATION
STATION #__________RIVERMILE ____________________	STREAM CLASS
LAT_______________LONG ____________________	RIVER BASIN
STOREET #	AGENCY
INVESTIGATORS	
FORM COMPLETED BY	DATE ________ TIME ________ AM PM REASON FOR SURVEY

Para	Habitat Parameter	Condition Category			
		Optimal	**Suboptimal**	**Marginal**	**Poor**
	1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and <u>not</u> transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 87 6	5 4 3 2 1 0
	2. Embeddedness	Gravel, cobble, and boulder particles are 0 25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25 50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50 75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 87 6	5 4 3 2 1 0
	3. Velocity/Depth Regime	All four velocity/depth regimes present (slow deep, slow-shallow, fast- deep, fast-shallow). (Slow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 87 6	5 4 3 2 1 0

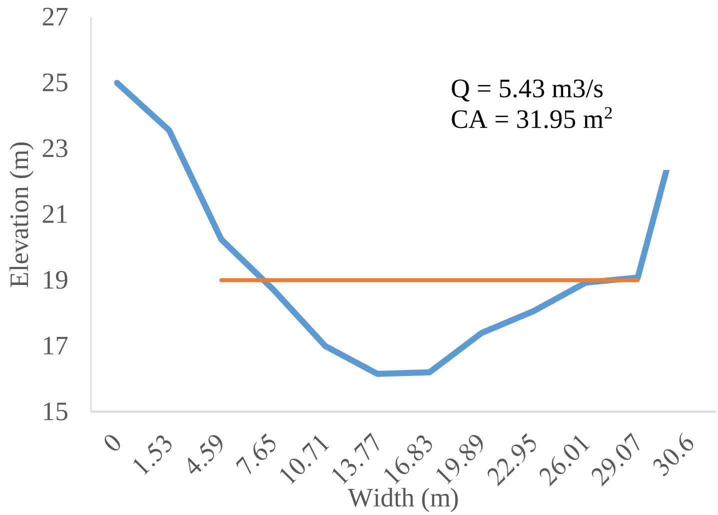
	4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 87 6	5 4 3 2 1 0
	5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 87 6	5 4 3 2 1 0

	Habitat Parameter	**Condition Category**			
		Optimal	**Suboptimal**	**Marginal**	**Poor**
	6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
P	**7. Frequency of Riffles (or bends)**	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	8. Bank Stability (score each bank) Note: determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30 60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional

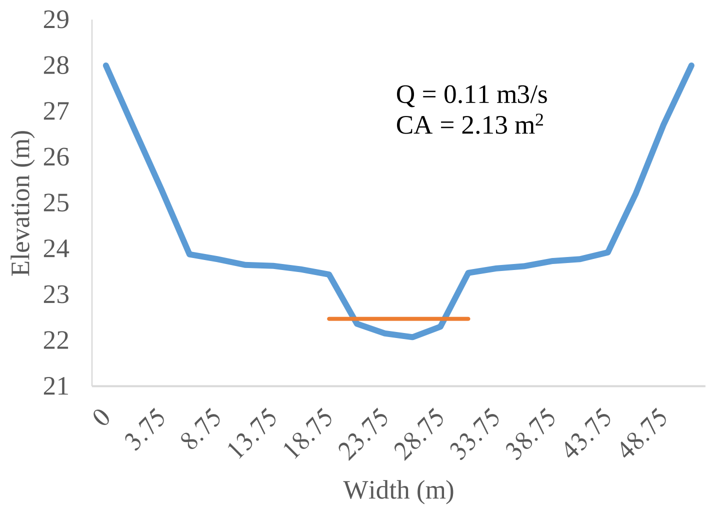
				scars.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well- represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one- half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6 12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE(RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

Total

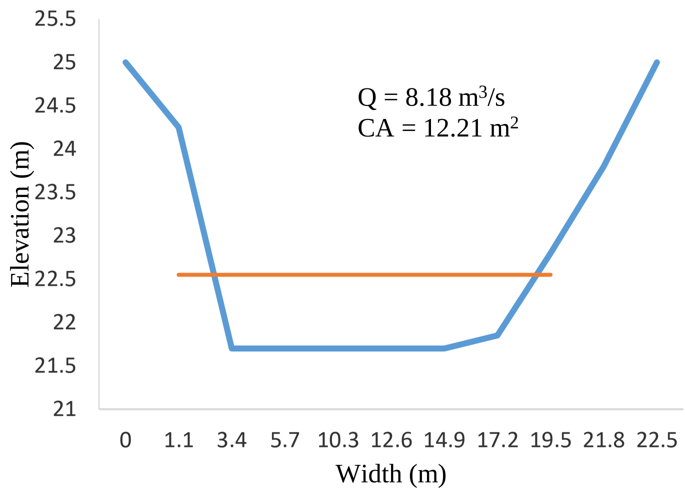
Appendix H



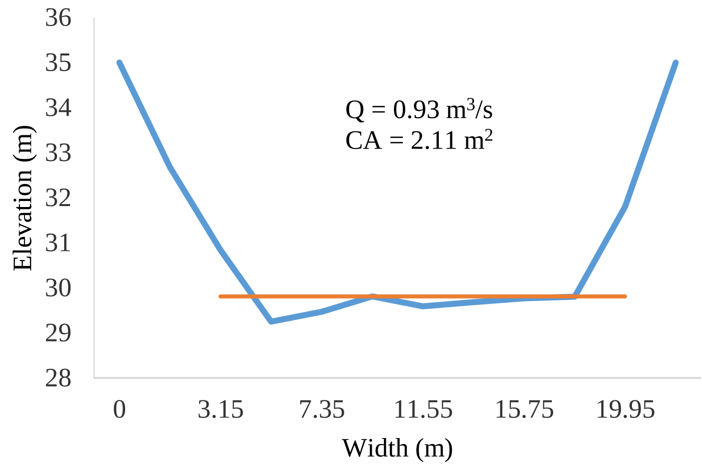
SR 1 : Gunthong River



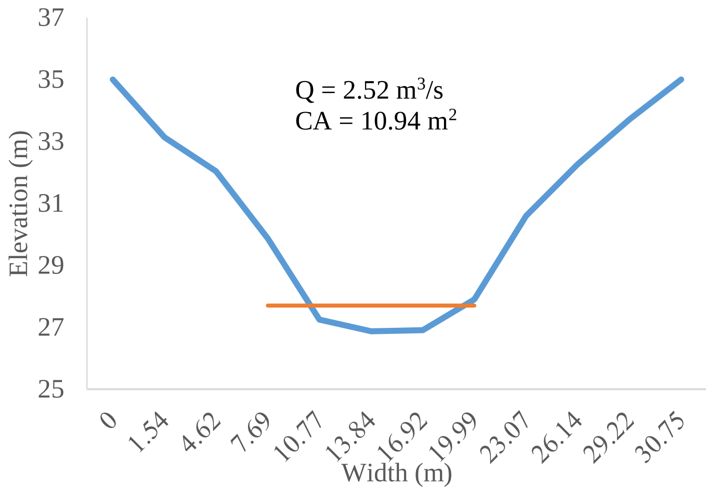
SR2 : Gunthong River tributary



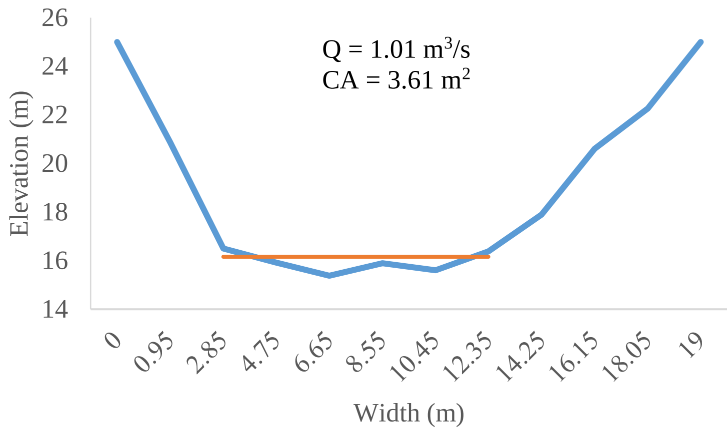
SR 3 : Kunag River



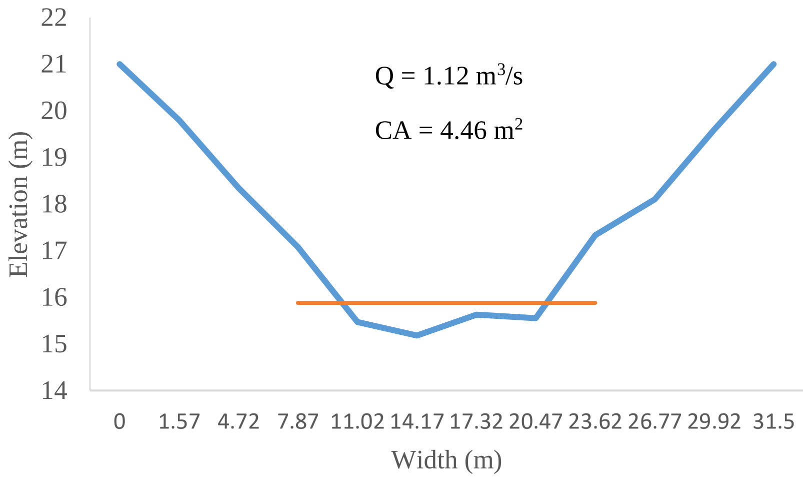
SR 4: Gong River



SR 5: Buaya River



SR 6: Kuang River



SR 7: Kuang River

Figure H1 : River cross sectional area (m^3/s) and discharge (m^3/s) for all the sampling sites, the Rawang sub-basin, Selangor River

Appendix I : Some macrobenthos in the study area identified

 *Turbificidae* Magnification: X400	 Hagenius *sp. (Gomphidae)* Magnification: X400
 *Caenidae* sp. Magnification: X400	 Brachydeutera *sp.* Magnification: X400

Atyidae
Magnification: X400

Amphipsyche *sp.*
Magnification: x400

Cordulia *sp.*
Magnification: x400

Prodasineura *sp.*
Magnification: x400

Nais *sp.*
Magnification: x50

Corbicula *sp.*
Magnification: x100

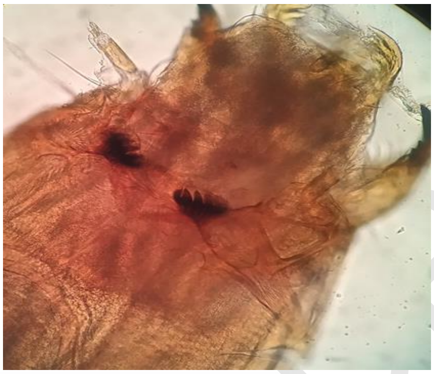
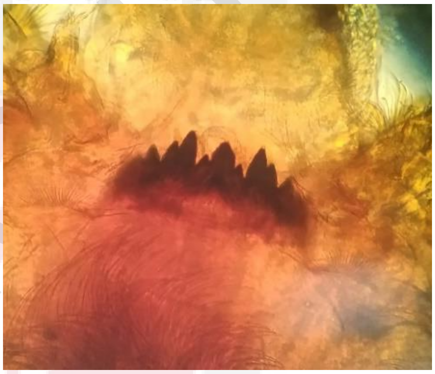
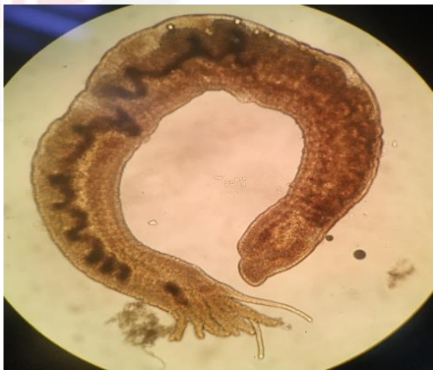
 *Tanypodinae* Magnification: x400	 Cryptochironomus *sp.* Magnification: x400
 *Polypedilum* sp. Magnification: x400	 Chironomus *sp.* Magnification: x400
 *Pristina* sp. Magnification: x400	 Aulophorus *sp.* Magnification: x400

Figure I1: Some macrobenthos in the study area identified

Appendix J

Table J1 : The Kruskal Wallis test outputs for the variation of the total macrobenthos compositions among sampling months

Test Statistics[a,b]				
Macrobenthos family	**Kruskal-Wallis H**	**df**	**Asymp. Sig.**	**Decision**
Total number of Macrobenthos	31.285	6	0.000	Reject null hypothesis
a:Kruskal Wallis Test				
b: Grouping Variable: Month				

Table J2 : The Kruskal Wallis test outputs for the variation of the total macrobenthos compositions among sampling months after trimmed out rare taxa

Test Statistics[a,b]				
Macrobenthos family	**Kruskal-Wallis H**	**df**	**Asymp. Sig.**	**Decision**
Total number of Macrobenthos	31.113	6	0.000	Reject null hypothesis
a:Kruskal Wallis Test				
b: Grouping Variable: Month				

Table J3 : The Kruskal Wallis test outputs for the macrobenthos family level comparison among sampling months excluding the reference site

Test Statistics[a,b]				
Macrobenthos family	**Kruskal-Wallis H**	**df**	**Asymp. Sig.**	**Decision**
Naididae	47.600	6	0.000	Reject null hypothesis
Aeolosomatidae	14.919	6	0.021	Reject null hypothesis
Atyidae	11.148	6	0.084	Reject null hypothesis
Lumbriculidae	20.561	6	0.002	Reject null hypothesis
Tubificidae	4.722	6	0.580	Retain null hypothesis
Chironomidae	44.166	6	0.000	Reject null hypothesis
Hydropsychidae	23.659	6	0.001	Reject null hypothesis
Glossiphoniidae	33.947	6	0.000	Reject null hypothesis
Haplotaxidae	13.876	6	0.031	Reject null hypothesis
Baetidae	10.246	6	0.115	Retain null hypothesis
Viviparidae	8.003	6	0.238	Retain null hypothesis
Caenidae	5.040	6	0.539	Retain null hypothesis
Erpobdellidae	5.628	6	0.466	Retain null hypothesis
Corbiculidae	3.611	6	0.729	Retain null hypothesis
Libellulidae	4.959	6	0.549	Retain null hypothesis
Protoneuridae	18.796	6	0.005	Reject null hypothesis
Thiaridae	7.870	6	0.248	Retain null hypothesis
Unindentified Oligocheata	8.293	6	0.217	Retain null hypothesis
a:Kruskal Wallis Test				
b: Grouping Variable: Month				

Appendix K

Table K1 : The Kruskal Wallis test outputs for variation of water quality among sampling sites

Test Statistics[a,b]				
Water quality parameter	**Kruskal-Wallis H**	**df**	**Asymp. Sig.**	**Decision**
Water temperature	39.868	6	0.000	Reject null hypothesis
pH	23.990	6	0.001	Reject null hypothesis
DO	32.822	6	0.000	Reject null hypothesis
EC	17.566	6	0.007	Reject null hypothesis
Turbidity	9.914	6	0.128	Retain null hypothesis
Ammoniacal-N	28.857	6	0.000	Reject null hypothesis
BOD	9.670	6	0.139	Retain null hypothesis
COD	13.025	6	0.043	Reject null hypothesis
TSS	7.952	6	0.242	Retain null hypothesis

a:Kruskal Wallis Test
b: Grouping Variable: Month

Appendix L

Table L1 : The Kruskal Wallis test outputs for variation of water quality among sampling months

Test Statistics[a,b]				
Water quality parameter	**Kruskal-Wallis H**	**df**	**Asymp. Sig.**	**Decision**
Water temperature	39.868	6	0.000	Reject null hypothesis
pH	23.990	6	0.000	Reject null hypothesis
DO	32.822	6	0.000	Reject null hypothesis
EC	17.566	6	0.000	Reject null hypothesis
Turbidity	9.914	6	0.028	Reject null hypothesis
Ammoniacal-N	28.857	6	0.000	Reject null hypothesis
BOD	9.670	6	0.139	Retain null hypothesis
COD	13.025	6	0.043	Reject null hypothesis
TSS	13.025	6	0.043	Retain null hypothesis

a:Kruskal Wallis Test
b: Grouping Variable: Month

Appendix M

Table M1 : The Kruskal Wallis test outputs for variation of water quality among sampling months when excluding the reference site

Test Statistics[a,b]				
Water quality parameter	**Kruskal-Wallis H**	**df**	**Asymp. Sig.**	**Decision**
Water temperature	34.344	6	0.000	Reject null hypothesis
pH	32.531	6	0.000	Reject null hypothesis
DO	37.176	6	0.000	Reject null hypothesis
EC	25.374	6	0.000	Reject null hypothesis
Turbidity	14.168	6	0.028	Reject null hypothesis
Ammoniacal-N	38.012	6	0.000	Reject null hypothesis
BOD	16.386	6	0.012	Retain null hypothesis
COD	20.523	6	0.002	Reject null hypothesis
TSS	11.194	6	0.083	Retain null hypothesis

a:Kruskal Wallis Test
b: Grouping Variable: Month

Appendix N

Table N1 : The Kruskal Wallis test outputs for variation of WQI and sub-index among sampling sites

Test Statistics[a,b]				
Water quality Index and subindex	**Kruskal-Wallis H**	**df**	**Asymp. Sig.**	**Decision**
WQI	14.063	6	0.029	Reject null hypothesis
SIDO	30.445	6	0.000	Reject null hypothesis
SIBOD	8.656	6	0.194	Retain null hypothesis
SICOD	13.273	6	0.039	Reject null hypothesis
SIAN	29.650	6	0.000	Reject null hypothesis
SISS	7.546	6	0.273	Retain null hypothesis
SIpH	24.179	6	0.000	Reject null hypothesis

a:Kruskal Wallis Test
b: Grouping Variable: Month

Appendix O

Table O1 : The Kruskal Wallis test outputs for variation of WQI and sub-index among sampling months

Water quality index and sub-index	Kruskal-Wallis H	df	Asymp. Sig.	Decision
WQI	14.063	6	0.029	Reject null hypothesis
SIDO	30.445	6	0.000	Reject null hypothesis
SIBOD	8.656	6	0.194	Reject null hypothesis
SICOD	13.273	6	0.039	Reject null hypothesis
SIAN	29.650	6	0.000	Reject null hypothesis
SISS	7.546	6	0.273	Retain null hypothesis
SIpH	24.179	6	0.000	Reject null hypothesis

a:Kruskal Wallis Test
b: Grouping Variable: Month

Appendix P

Table P1 : The Kruskal Wallis test outputs for variation of WQI and sub-index among sampling months when excluding the reference site

Water quality index and sub-index	Kruskal-Wallis H	df	Asymp. Sig.	Decision
WQI	21.109	6	0.002	Reject null hypothesis
SIDO	36.423	6	0.000	Reject null hypothesis
SIBOD	14.814	6	0.022	Reject null hypothesis
SICOD	20.913	6	0.002	Reject null hypothesis
SIAN	38.190	6	0.000	Reject null hypothesis
SISS	11.477	6	0.075	Retain null hypothesis
SIpH	30.156	6	0.000	Reject null hypothesis

a:Kruskal Wallis Test
b: Grouping Variable: Month

Appendix Q

Table Q1 : National Water Quality Standards for Malaysia

Parameters	Unit	Class					
Ammoniacal-nitrogen	mg/l	**I**	**IIA**	**IIB**	**III**	**IV**	**V**
Biochemical Oxygen Demand	mg/l	0.1	0.3	0.3	0.9	2.7	>2.7
Chemical Oxygen Demand	mg/l	1	3	3	6	12	>12
Dissolved Oxygen	mg/l	7	5-7	5-7	3-5	<3	<1
pH	-	6.5 – 8.5	6-9	6-9	5-9	5-9	-
Colour	TCU	15	150	150	-	-	-
Electrical Conductivity*	µS/cm	1000	1000	-	-	6000	-
Floatables		N	N	N	-	-	-
Odour		N	N	N	-	-	-
Salinity	%	0.5	1	-	-	2	-
Taste	-	N	N	N	-	-	-
Total Dissolved Solid	mg/l	500	1000	-	4000	-	-
Total Suspended Solid	mg/l	25	50	50	150	300	300
Temperature	^{O}C	-	Normal + 2 ^{O}C	-	Normal + 2 ^{O}C	-	-
Turbidity	NTU	5	50	50	-	-	-
Faecal Coliform**	count/100 ml	10	100	400	5000 (20000)a	5000 (20000)a	-
Total Coliform	count/100 ml	100	5000	5000	50000	50000	>50000

Notes
* = At hardness 50 mg/l CaCO3
# = Maximum (unbracketed) and 24–hour average (bracketed) concentrations
N = Free from visible film sheen, discolouration and deposits

Appendix R

Table R1 : Summary of the multiple linear regression for Group 1 (Haplotaxidae, Lumbriculidae, Naididae, and Unidentified Oligochaeta)

Name of the Model	MLR used	Dependent Variable	Variable Selected from MLR	R^2	Adjusted R2	Significant value	Standardized Coefficients Beta	Regression Model	Significant status of each individuals variable
Model 1	Backward	Haplotaxidae	-	-	-	*P*>0.05 Not Significant	-	-	-
Model 2	Backward	Lumbriculidae	NH_3 DO COD	0.120	0.098	*P*<0.05 (Sig: 0.01) Significant	NH_3: 0.278 DO: 0.442 COD: 0.314	Y = - 25.176 + 3.214 NH_3 + 2.921 DO + 0.345 COD	Constant :0.006 NH_3: $p<0.05$ DO: $p<0.05$ COD: $p<0.05$
Model 3	Backward	Unidentified Oligochaeta	-	-	-	*P*>0.05 Not Significant	-	-	-
Model 4	Backward	Naididae	NH_3	0.059	0.052	*P*<0.05 (0.006) Significant	NH_3: 0.243	Y = 2.365 + 5.458 NH_3	Constant: 0.458 NH_3: p<0.05

Table R2 : Summary of the multiple linear regression for Group (Libellulidae and Protoneuridae)

Name of the Model	MLR used	Variable	Variable Selected from Stepwise MLR	R^2	Adjusted R2	Significant value	Standardized Coefficients Beta	Regression Model	Significant status of each individuals variables
Model 1	Backward	Libellulidae	TSS	0.149	0.142	$P<0.05$ (0.000) Significant	TSS: 0.386	Y = 0.037 + 0.000 TSS	Constant: 0.037 TSS: $p<0.05$
Model 2	Backward	Protoneuridae	TSS	0.126	0.112	$P<0.05$ (0.000) Significant	NH_3:0.149 TSS: 0.340	Y = -0.178 + 0.097 NH_3 + 0.02 TSS	Constant :0.000 NH_3: $p>0.05$ TSS: $p<0.05$

Table R3 : Summary of the multiple linear regression for Group 3 (Viviparidae, Corbiculidae, and Thiaridae)

Name of the Model	MLR method used	Variable	Variable Selected from Stepwise MLR	R^2	Adjusted R2	Significant value	Standardized Coefficients Beta	Regression Model	Significant status of each individuals variables
Model 1	Backward	Viviparidae	-	-	-	*P*>0.05 Not significant	-	-	-
Model 2	Backward	Corbiculidae	NH_3	0.043	0.035	*P*<0.05 (0.020) Significant	NH_3: -0.207	Y = 0.226 – 0.103 NH_3	Constant :0.002 NH_3: $p<0.05$
Model 3	Backward	Thiaridae	-	-	-	*P*>0.05 Not significant	-	-	-

Table R4 : Summary of the multiple linear regression for Group 4 (Baetidae Caenidae, Hydropsychidae and Atyidae)

Name of the Model	**MLR method use**	**Variable**	**Variable Selected from Stepwise MLR**	**R^2**	**Adjusted R2**	**Significant value**	**Standardized Coefficients Beta**	**Regression Model**	**Significant status of each individuals variables**
Model 1	Backward	Baetidae	BOD, COD, DO	0.091	0.069	$P<0.05$ (0.009) Significant	BOD: -0.347 COD: 0.539 DO: 0.283	Y = -6.290 - 0.760 BOD + 0.208 COD + 0.656 DO	Constant: 0.069 BOD: $p>0.05$ COD:$p<0.05$ DO: $p>0.05$
Model 2	Backward	Caenidae	-	-	-	$P>0.05$ Not significant	-	-	-
Model 3	Backward	Hydropsychidae	DO and COD	0.078	0.063	$P<0.05$ (0.007) Significant	DO: 0.433 COD: 0.262	Y = -11.103 + 0.153COD + 1.520 DO	Constant :0.020 DO: $p<0.05$ COD: $p>0.05$
Model 4	Backward	Atyidae	-	-	-	$P>0.05$ Not significant			

Table R5 : Summary of the multiple linear regression for Chironomidae and Tubificidae

Name of the Model	MLR used	Dependent Variable	Variable Selected from MLR	R^2	Adjusted R2	Significant value	Standardized Coefficients Beta	Regression Model	Significant status of each individuals variables
Model 1	Backward	Chironomidae	DO NH_3 TSS	0.182	0.162	$P<0.05$ (Sig: 0.000) Significant	DO: 0.311 NH_3: 0.381 TSS: 0.236	$Y = -31.179 + 5.360\ DO + 11.508\ NH_3 + 0.071\ TSS$	Constant :0.003 DO: ($p<0.05$) NH_3: ($p<0.05$) TSS: ($p<0.05$)
Model 2	Backward	Tubificaide	BOD DO	0.139	0.125	$P<0.05$ (0.000) Significant	BOD:0.641 DO: 0.392	$Y = -137.636 + 22.472\ BOD + 14.552\ DO$	Constant: 0.005 BOD: (p<0.05) DO: (p<0.05)

BIODATA OF STUDENT

Nadeesha Dilani Hettige was born in Beliatta, Sri Lanka. She attended both primary and secondary school in Debarawewa Primary school and Vishaka Balika Madya Maha Vidyalaya, Bandarawela, Sri Lanka. She proceeded to the Sabaragamuwa University of Sri Lanka, where she obtained a Bachelor of Applied Science (Special) in Environmental Science and Natural Resource Management with a first class. She has also received the Master of Science in Environmental Science from the University of Peradeniya, Sri Lanka. She has obtained a Ph.D. scholarship from Sri Lanka Council for Agricultural Research Policy (SL CARP) – 2018. After that, she further her studies in Doctor of Philosophy in Marine and Freshwater Ecosystem under the supervisor of Dr. Rohasliney binti Hashim in Universiti Putra Malaysia. She has 11 years' experience as a scientist at Environmental Studies Division, National Aquatic Resource Research and Development Agency (NARA), Sri Lanka.

LIST OF PUBLICATIONS

Journals

Hettige, N.D., Hashim, R. B., Kutty, A. B. A., Ash'aari, Z. H. B. & Jamil, N.R.B. (2022). 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, Journal of Fisheries and Environment. *46*(1), 180-197. **Published** (SCOPUS)

Hettige, N.D., Hashim, R. B., Kutty, A. B. A., Jamil, N.R.B. & Ash'aari, Z. H. B. (2020). Application of ecological indices using macroinvertebrate assemblages in relation to aquaculture activities in Rawang Sub-basin, Selangor River, Malaysia, *Pertanika Journal Science & Technology, 28* (S2), 25-45. **Published** (SCOPUS)

Hettige, N. D., Rohasliney, H., Ashaari, Z. H. B., Kutty, A. B.A., & Jamil, N. R. B. Assessment of physical health characteristics in selected Selangor Rivers in Malaysia. *Songklanakarin Journal of Science and Technology.* **Under review.** (SCOPUS)

International Conference and Symposium

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Hettige, N. D., Rohasliney, H., Ashaari, Z. H. B., Kutty, A. B.A., & Jamil, N. R. B. (2020). Application of GIS for water quality monitoring in the aquaculture impacted Rawang sub-basin of the Selangor River, Malaysia. *Proceeding of 10th Annual International Conference 2020 on Environmental and Life Science* (p.23). Banda Aceh, Indonesia: Universitas Syiah Kauala. **Published.**
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Hettige, N.D., Hashim, R.B., Kutty, A. B. A., Jamil, N. R. B. & Ash'aari, Z. H. B. (2019). Application of ecological indices in determining the Benthic macroinvertebrates diversity in Rawang Sub-basin, Selangor River, Malaysia: Preliminary study. *Proceeding of 7th International Symposium on Applied Engineering and Sciences* (p. 67). Serdang, Selangor, Malaysia: Faculty of Engineering, Universiti Putra Malaysia. **Published.**