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### POTENTIAL USE OF FRESHWATER INSECT LARVAE IN RIVER BIOLOGICAL MONITORING: A SYSTEMATIC REVIEW

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#### ABSTRACT

Freshwater insect larvae, also known as macroinvertebrates, are potential bio-indicators for water quality monitoring in Malaysia, as they were reported to have extensive density and distribution in Malaysian river ecosystems. The indices formed based on macroinvertebrate biogeographical data exhibited a good conjunction with the national water quality standard (NWQS) and water quality index (WQI) for monitoring the water quality in Malaysia. With a significant sensitivity, macroinvertebrates provide usable responses to environmental changes, which are highly similar to the water quality indices. Even these indices can provide an assessment of river quality faster and cheaper. This systematic study was conducted to demonstrate the potential use of macroinvertebrate-based available indices. Selected features evaluated are taxonomy level, type of indices, study area, and the similarity of the result with WQI. The survey was conducted using the Systematic Reviews and Meta-Analyses (PRISMA) system consisting of eight stages of analysis. Manuscript searches have been conducted using Scopus and Web of Science search engines. Searched manuscripts were filtered according to the scope of the study and only manuscripts that meet the inclusion criteria were selected. A total of 184 manuscripts were found throughout the search and only 36 manuscripts were accepted for further analysis. Subsequently, the extraction of information from selected manuscripts was carried out, and each chosen issue's division was arranged in Microsoft excel. This study found that the most widely used biotic indices in Malaysia were Biological Monitoring Working Party (BMWP) followed by Average Score Per Taxon (ASPT), and the Family Biotic Index (FBI), while the most used diversity indices were the Shannon diversity index (H') followed by the Margalef richness index (R') and the Pielou evenness index (J). The family level was the most frequent for indices calculations compared to genus and species. Among all the indices used, BMWP was statistically proven to have the closest result to WQI, followed by the FBI, H' and R'. The high similarity value with WQI proves that the macroinvertebrate's biotic and diversity indices are very significant in monitoring river ecosystem quality in Malaysia even if only at the family taxonomic level. These indices also showed small variations even in different rivers, demonstrates the suitability of many types of rivers in Malaysia.

Keywords: Freshwater, Biological indicator, Water quality index, Biotic indices, Diversity indices

#### ABSTRAK

Larva serangga akuatik atau dikenali juga sebagai makroinvertebrat merupakan penunjuk biologi untuk pemantauan kualiti air di Malaysia, kerana ia dilaporkan mempunyai kepadatan dan sebaran yang tinggi dalam ekosistem sungai di Malaysia. Indeks yang dibentuk berdasarkan data biogeografi menunjukkan kesamaan yang baik dengan piawaian kualiti air kebangsaan (NWOS) dan indeks kualiti air (WOI) untuk pemantauan kualiti air di Malaysia. Dengan sensitiviti yang tinggi, makroinvertebrat memberi respon yang digunapakai terhadap perubahan alam sekitar dan respon ini mempunyai kesamaan yang tinggi dengan indeks kualiti air. Malah indeks biotik ini dapat memberikan penilaian yang lebih cepat dan murah. Kajian sistematik ini dijalankan untuk menujukkan potensi penggunaan makroinvertebrat berdasarkan kepada indeks yang tersedia. Kriteria yang dinilaikan adalah aras taksonomi, jenis indeks, kawasan kajian dan kesamaan hasil indeks biotik terhadap keputusan indek WQI. Tinjauan telah dilakukan menggunakan sistem Kajian Semula dan Meta-Analisis (PRISMA) yang mengandungi lapan peringkat analisis. Pencarian manuskrip telah dilakukan menggunakan Scopus dan Web of Science. Hasil carian telah ditapis untuk memenuhi skop kajian dan hanya manuskrip yang memenuhi ciri pilihan sahaja yang dipilih. Sejumlah 184 manuskrip telah ditemui dan hanya 36 manuskrip sahaja yang telah diterima untuk tindakan lanjutan. Seterusnya maklumat daripada manuskrip pilihan di saring dan disusun mengikut skop dalam format Microsoft Excel. Kajian ini mendapati indeks yang paling kerap digunakan adalah Biological Monitoring Working Party (BMWP) diikuti oleh Average Score Per Taxon (ASPT) dan indeks Family Biotic Index (FBI) manakala indeks kepelbagaian yang paling kerap digunakan adalah indeks kepelbagaian Shannon (H') diikuti oleh indeks kekayaan Margalef  $(R_{Mg'})$  dan indeks keseragaman Pielou (J). Aras taksonomi famili paling banyak digunapakai berbanding genus ataupun spesies. Dikalangan semua indeks yang digunakan, indeks BMWP terbukti secara statistic memberikan keputusan yang paling mirip dengan indeks WQI, diikuti oleh FBI, H' dan R<sub>Mg</sub>'. Kesamaan keputusan yang tinggi dengan WQI membuktikan bahawa indeks biotik dan kepelbagaian makroinvertebrat bentik adalah sangat bermakna untuk digunakan dalam pemonitoran sungai di Malaysia walaupun hanya pada aras taksonomi famili. Indeks ini menunjukkan variasi nilai yang kecil wapaupun pada sungai yang berbeza yang menunjukkan kesesuaian untuk digunakan dalam sebarang jenis sungai di Malaysia.

Kata kunci: Ekosistem Air Tawar, Penunjuk Biologi, Indek Kualiti Air, Indek Biotik, Indek Kepelbagaian

#### **INTRODUCTION**

Anthropogenic activities and human disturbance can cause deterioration of water quality and threaten the river's ecosystem. Insect larvae which represent macroinvertebrates benthic in river systems, are known for their capability to react, respond, or adapt to any changes in the environment, either as an individual or community (Abas 2021; Sabina Noor et al. 2021). Due to their broad distribution, high abundance, short life cycle, and rapid response to anthropogenic pressure (Castillo-Figueroa et al. 2018; Kutty et al. 1999), macroinvertebrates benthic are useful bioindicators for the water quality assessment (Amila & Suhaila 2019; Ng & Nurul Afiqah 2023; Muhammad Adam & Amiruddin 2024; Suhaila et al. 2018). There is a growing interest in biomonitoring studies in many countries such as the United States of America, Australia, Europe and Asia, resulting in many diversity and biotic indices developments. Among widely used indices include Biological Monitoring Working Party (BMWP), Average Score Per Taxon (ASPT), Family Biotic Index (FBI) dan Ephemeroptera, Plecoptera, and Trichoptera Index (EPT). Some countries may adapt those indices based on

their similarity in geographical region, taxonomy found, and climate changes. The difference in these factors could affect the presence and type of taxonomy found in the aquatic ecosystem and therefore been modified to suit their region characteristics.

Malaysia still uses water quality indices for river monitoring purposes which are the Water Quality Index (WQI) and the National Water Quality Standard (NWQS). These methods are derived from the measurement of the physicochemical parameters of the water (Ghani 2016). However, taking into consideration the physicochemical aspects alone is not sufficient to indicate ecosystem health as a whole (Salmiati & Salim 2017) as it does not provide insight into the effects of environmental pollution on habitat and aquatic life. Thus, biological assessment should be carried out simultaneously with the physicochemical method to achieve a comprehensive assessment of the water bodies (Salmiati & Salim 2017). Nowadays, studies on biomonitoring using macroinvertebrates benthic as a bioindicator have been rapidly increasing in Malaysia. Most of the studies used an integrated method of physicochemical along with biological assessment to evaluate the water quality (Ab Hamid & Rawi 2017; Al-Shami et al. 2011; Ahmad et al. 2021; Hettige et al. 2020; Nurhafizah-Azwa et al. 2018; Sabina Noor et al. 2021). The growing interest among local researchers could bring a positive sign for the development of biological indices for monitoring programs in Malaysia.

However, with the number of studies that have been conducted, the information could be dispersed and is often difficult to find. Only a few attempts have been made to critically evaluate and synthesize the findings from these many studies. Therefore, this systematic review is conducted to provide insightful information regarding the potential use of macroinvertebrates benthic indices in the biological monitoring program in Malaysia. This study also aims to analyse the research that has been conducted based on studied parameters, study area, and type of biological and diversity indices being used. Integration research information on Malaysia invertebrate's role will be useful to evaluate the progress of biomonitoring, define priority in future research, define the weakness for current indices used, and identify the gaps in terms of biomonitoring using macroinvertebrate benthic. This systematic review is also important in establishing a benchmark for the benthic macroinvertebrate index for the future development of biological monitoring programme in Malaysia.

### SEARCH STRATEGY

A comprehensive search for data has been made through search engines and databases Scopus, ScienceDirect, Web of Science, Google Scholar, and ResearchGate without restricting publication year. The articles were searched using related keywords with all the possible combinations: (*"macroinvertebrates"* OR *"environmental indicator"* OR *"macroinvertebrates"* OR *"macroinvertebrates"* OR *"aquatic macroinvertebrates"*) AND (*diversity"* OR *"distribution* OR *"water quality"*). To narrow down the search process, the keywords for the countries associated with the study also be used such as (Malaysia" OR *"Thailand"* OR *"Vietnam"*). The study was conducted referring to the guideline Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al. 2009) but the Meta-Analysis method would not be included.

### **Inclusion and Exclusion Criteria**

The articles that were included were primarily studied in English or Malay language. The selected article must include the usage of macroinvertebrates benthic for various study objectives such as water quality and habitat assessment. The study area was focused on Malaysia and restricted to the freshwater ecosystem such as rivers, streams, and tributaries.

Articles were excluded if the sources were from books, reports, or systematic review studies (journal only). Besides, some articles also were rejected due to their incompatibility with this study's aims, such as articles that studied biomonitoring other than invertebrates. Lastly, the articles selected also must present the clear usage of both macroinvertebrates' benthic indices.

## SCREENING DATA

During the screening process, the duplicate articles from various databases were eliminated. The abstract part was used to assess duplication and those articles that contain similar abstracts will be eliminated. After the full text was downloaded, the articles were filtered once again to ensure that the selected articles provided the right information regarding the study. The final selection of the systematic review consisted of 36 articles.

# DATA ANALYSIS

To quantify data, a Microsoft excel database was compiled in which the following attributes were included such as spatial and temporal distribution that includes the year of publication, type of study area and distribution of article published. The context of the article that is extracted such as the type of indices used, level of taxonomy and the usage of WQI were evaluated statistically. The analysis of data includes descriptive statistical analysis and inferential statistical analysis such as correlation test.

# **Temporal and Spatial Distribution**

The total number of articles reviewed is shown in Figure 1. Benthic macroinvertebrates have received great concern recently for river assessment and monitoring in Malaysia and the reported manuscript gradually increased within 21-year period (Figure 1). The highest publication related to macroinvertebrates indices in biological monitoring program was recorded in 2018 (17%) while the lowest resulted from 1999 to 2010. The earliest study was conducted by Yap (1997) regarding the classification of Malaysian's rivers using biological indices and Kutty et al. (1999) on the study of biological monitoring using macroinvertebrates at Sungai Langat, Selangor, and in 2020 by Dhiya Shafiqah et al. (2020) for monitoring aquatic insects influenced by season. The number of articles gradually increased reflecting research interest by the researchers. This increase is more evident in the period 2015-2020 where more than 60% (n=22) of total articles were reported in this period. Although it fluctuated from the year 2019 onwards, the increased trendline (r=0.67) shown in Figure 1 indicates an expectation that more articles would be published in the future. However, the covid-19 pandemic outbreak would restrict the expectations.

Figure 2 shows the total number of articles according to their respective states in Malaysia. Most of the studies were conducted in Johor and Terengganu with 7 articles (19%) and 5 articles (14%) respectively followed by Perak and Kelantan with 3 articles (8%), Sabah with 2 articles (6%) while Selangor, Kedah, and Pahang are 4 articles (11%). Only 1 article was reported in Penang, Negeri Sembilan, Sarawak and Kuala Lumpur. However, through searching from search engines like Scopus and Web of Science, there is no publication reported for Perlis and Melaka.



Figure 1. Temporal pattern for article publications for a 21-years period



Figure 2. Number of articles by states in Malaysia

# **Study Locations**

Thirteen articles (36%) studied biomonitoring at pristine forest rivers, followed by exposed basin areas and non-virgin forest areas where both areas had six articles (17%). Only five articles (14%) reported biomonitoring in urban rivers and four articles (11%) at high land areas (Figure 3). The least study of invertebrates' biomonitoring was recorded in the island area with only two articles (5%) published. In this study, the pristine areas relating to protected forest areas include forest reserves, recreational forests, education forests and national park forests. These areas are facing restricted development, agriculture or any kind of activity that would require the plants to be removed. Many studies have been conducted here because invertebrates are present with high diversity and density, especially sensitive groups such as Ephemeroptera, Plecoptera, Trichoptera order (EPT) that are known for being the best bioindicator in water quality monitoring due to their high sensitivity towards any environmental changes.

Coleoptera, Odonata and Diptera also get the attention of most researchers because of its widespread distribution and are listed in most indices.

On the other hand, a freshwater ecosystem in the urban area is also chosen as the study area by the local researchers. Rivers in urban areas mostly experience a significant decrease in quality as a result of anthropogenic activities and have benthos composition and diversity different from pristine areas. The water quality also degraded due to the inflow of wastes and effluents from the industries and settlement areas. This can affect the health of the river and aquatic life eventually. Hence more tolerant group invertebrates are present in this ecosystem, and biomonitoring studies are capable of detecting the degradation of river quality.



Figure 3. The spatial of invertebrates' biomonitoring study

# **APPLICATION OF INDICES**

### **Biotic indices**

Various types of biotic indices have been used for the biological monitoring programme in Malaysia. The BMWP index was the most frequently used (50%) followed by ASPT, FBI, and EPT with 39%, 31% and 28% respectively (Figure 4). On the other hand, the BMWP Thai index was used by local researchers for the river assessment with 11%. The least used biotic indices are the SIGNAL and Lincoln quality index, which were only 6% each.

BMWP index became the most popular index due to its easy application and frequently was complemented by ASPT index. The taxonomic resolution of the BMWP index is at the family level and this will make the identification process easier as available information at the genera or species level is scarce. The BMWP index initially was formed for the temperate

region environment. Therefore, to achieve a more accurate estimation, the modification of the original BMWP is recommended to suit with local region. The difference in climate and geography could affect habitat suitability and taxa presence. Thailand has been ahead of other countries in Southeast Asia by developing and established its own BMWP index called BMWP<sup>Thai</sup>. The index was a modified version of BMWP with some addition, elimination, and regroup of macroinvertebrates according to local taxa (Zakaria & Mohamed 2019). As Malaysia is located within a similar climatology territory with Thailand, the usage of BMWP<sup>Thai</sup> in biological monitoring would provide better results for the Malaysia river water assessment. Despite the low number of articles that use BMWP<sup>Thai</sup> in the study, there is a potential for the index to be widely used in the future as local researchers begin to compare several indices including BMWP<sup>Thai</sup> with the original BMWP to determine which biotic indices have better reflected on health of the aquatic ecosystem in Malaysia. Zakaria & Mohamed (2019) reported that to date, BMWP<sup>Thai</sup> is the best index that reflects Malaysian's river condition compared to the original BMWP and other indices.



Figure 4. Proportion of biotic indices used

The second-highest index used is the Average Score Per Taxon (ASPT). The index can determine the average sensitivity of the families by dividing the BMWP score by the number of taxa present (Armitage et al. 1983). Seasonal changes in temperate countries affect benthos diversity and cause BMWP values to be unbalanced throughout the year. By dividing the BMWP value by the number of taxa present, the ASPT value provides a more realistic assessment. In addition, score allocated for the BMWP index was based on the most pollution-tolerant species within each family (Walley et al. 1997). Thus, they were not representative of the family as a whole (Paisley et al. 2014). The usage of ASPT can reduce the random error due to subjective derivation of individual scores as ASPT does not depend on species richness. Hence it is advisable to use the BMWP and ASPT together (Walley et al. 1998).

### **Diversity indices**

Based on Figure 5, the most frequently used diversity index is the Shannon diversity index (H') with 24 articles (67%), followed by the Pielou evenness index (J) and Margalef richness index (R') with 19 articles (53%) and 18 articles (50%) respectively. Some articles use different types of diversity indexes such as Simpson's diversity index (1-D) and Hills's diversity index (D) with five articles (14%) and three articles (8%) respectively. About 75% of the total articles used diversity indices in biological monitoring to determine the distribution, abundance, and composition of macroinvertebrates benthic. Some articles reported diversity indices alone because they only wanted to estimate stress levels based on the macroinvertebrate's assemblage, but few articles integrate diversity indices and biotic indices for water quality assessment. Shannon diversity receives a good usage rate due to easy calculation and provides more reliable results. The index is based on randomness present at a site and assumes both species richness and evenness are equal in distribution. On the other hand, the 1-D is a measure of diversity that considers the number of species present, as well as the dominance of each species. H' is preferred compared to others because of not being biased towards the dominant species. The number of articles that use the J and R' is not much different because most of the articles use these indices together with the Shannon diversity index. Even though the indices like H' combine richness and evenness components into a single measure, it is usually more informative to evaluate richness and evenness independently.



Figure 5. Proportion of diversity indices used

Nevertheless, measuring diversity in an ecosystem was a useful method for describing community structure, but it was not useful for determining the pollution level of water bodies (Kalyoncu et al. 2011). Different taxa of macroinvertebrates may have different tolerance levels to different types of biotic and abiotic factors. Results from the diversity index only tell us about the stress level of the water body, where low diversity expresses a high level of stress while high diversity expresses a low level of stress detected. Hence, the community structure of macroinvertebrates has commonly been used as an indicator of aquatic ecosystem conditions (Friberg et al. 2006; Ortiz & Puig 2007). A combination of diversity index and biotic indices can provide information on pollution status and at the same time could exhibit its effect on

community structure. Moreover, the biotic indices also not affected by the abundance of certain individuals because they only involve the score calculation at the family level (Ahmad et al. 2013). The score assigned is subjective and only describes the state of the water body in general. Hence the usage of biotic indices is advisable to be used along with the diversity indices in the aquatic ecosystem that show the sign of domination. 11 out of 36 articles assessed river quality using diversity index and WQI. The correlation test shows that H' and R' provide results that are in line with WQI with correlation values of 0.62 and 0.65 respectively (Table 1). Only the Pielou uniformity index does not correlate positively with WQI. In a state of high WQI value (clean ecosystem), there will be some intolerant taxa that will dominate the ecosystem and lower the uniformity value of Pielou. A clear description should be made of this index is included in the evaluation so as not to be misunderstood.

Table	The result of the correlation test between diversity indices and WQI		
	Shannon Diversity Index (H')	Margalef Richness Index (R')	Pielou Evenness Index (J)
WQI	0.621	0.654	-0.386

# LEVEL OF TAXONOMY

Figure 6 shows family level was the most frequently used and reported in 24 articles (67%) from the total 36 articles. This was followed by genus level with 8 articles (22%) and the least used taxonomy level was species where only four articles (11%). The family level received great concern from researchers with the high application of biotic indices such as BMWP and ASPT index. The limitation of a lower taxonomic key and difficulty in species identification makes family as preferable taxonomic level. In addition to that, the use at the family level gives results that are not much different from the lower level, making the family level an option in biological monitoring. Thus, the family level is accepted in river quality assessment due to low effort for identification, equivalent results with physio-chemical and capable of providing faster results. Thus, the usage of biological water quality monitoring techniques using macroinvertebrates family level is preferable and capable of detecting disturbance events after their occurrence (Hellawell 2012). The result also reflects the overall condition of the water body and provides a trend in water quality for a long period.



Figure 6. Level of taxonomy choices

On the other hand, there are still a few studies that identify the individuals at the species level. Theoretically, the use of lower taxonomic units such as species or genus provides more accurate information in assessing ecological conditions (Bouchard Jr et al. 2005). Some researchers argue that the usage of higher taxonomic groupings such as family may contain similar ecological traits and the result may not represent the whole condition of the environment. Different species from the same family may have different tolerance levels to the same stressor. Therefore, grouping them may lead to a wrong decision. However, identifying the organism at the species level is not practical for the biological monitoring process. Specieslevel identifications are often avoided for biological reasons, such as the very small size of aquatic invertebrates, limitations of taxonomic resources, availability of expertise, intensive sample preparation, and the allocation of limited time and resources (Ammann et al. 1997; Chessman et al. 2004; Feminella 2000). Moreover, finer taxonomic resolutions can decrease the accuracy of identification due to the requirement of some level of expertise (Stribling et al. 2008). Identification at the family level is very much easier than genus or species because it does not require specific identification skills. The difficulty of finer-resolution identification imparts a degree of error as more likely it will be incorrect and differ among laboratories and taxonomists (Bouchard Jr et al. 2005). Hence, coarser taxonomic resolutions would be the simplest way for the identification of aquatic macroinvertebrates (Bouchard Jr et al. 2005). The good indices are simple, fast, user user-friendly with an appropriate degree of accuracy, therefore the use of family level is more relevant than lower taxonomy.

Malaysia should consider the idea of creating its biotic indices based on taxonomy found specifically in Malaysia's aquatic ecosystem. Since the BMWP index was originally developed in the UK, the presence of aquatic macroinvertebrate communities might be varied due to the difference in geographic region and climate. For a better option, the local researchers can use BMWP-Thai in the biological monitoring method because the BMWP-Thai index possesses the closest taxonomies to Malaysia's. However, there are few taxonomies found in Malaysia but absent in Thailand's aquatic ecosystem. Hence, by creating Malaysia's biotic index in the future, the index will better reflect the health of the river system compared to the current use of indices such as BMWP and FBI. Furthermore, the study of macroinvertebrates benthic at lower taxonomic resolution also needs to be done especially on families that show a very wide tolerance range such as Chironomidae. The tolerance range will be narrow at the lower taxonomic level and this will provide a more accurate result (Jones 2008; Kutty et al. 2019).

### CONCLUSION

Through this study, macroinvertebrates benthic were seen to potentially be used as a bioindicator in biological monitoring programs in Malaysia. This can be shown by the correlation test conducted between biotic and diversity indices with WQI which show a strong positive relationship between them. The biological monitoring method should be considered as a complementary approach for stream water quality in Malaysia along with WQI, to achieve accurate and thorough results. Hence, the parallelism and relationship between bioindicator and WQI established in Malaysia should be further studied to fully understand the science between these 2 different methods. More research on biological monitoring could provide evidence for effectiveness in assessing stream water quality. Based on the data obtained, the taxonomy at the family level is widely being used in the assessment of aquatic ecosystems compared to genus and species due to its easy application. However, this study is limited to the freshwater ecosystem only, hence further studies should be conducted in a lentic ecosystem

such as lakes, swamps and reservoirs as many macroinvertebrates can be found in these ecosystems.

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## **AUTHORS DECLARATIONS**

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### **Conflict of Interest**

The authors declare that they have no conflict of interest

### **Ethics Declarations**

No ethical issue is required for this research.

### **Data Availability Statement**

This is a Final Year Project (FYP) and the data are currently in thesis of Hani Nazirah Suhaimi (2021).

### **Authors' Contributions**

Both authors were involved in drafting this SLR manuscript and joined forces for the writing and revision of the manuscript.

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