

## A Benthic Macroinvertebrate Multimetric Index for Assessment of the Ecological Integrity of Northeast Streams, Thailand

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### Abstract

This study aims to develop a benthic macroinvertebrate multimetric index for assessing the ecological quality of streams in Northeastern Thailand. ANOSIM indicated that the benthic macroinvertebrate assemblage in both of each basin and each season were not significantly different ( $R = 0.09$ ,  $p = 0.24$  and  $R = 0.07$ ,  $p = 0.35$ , respectively). The efficacy metrics of each basin consisting of the Mekong II, the Chi, and the Mun basins were integrated and calibrated. A total of 255 data sets of water physico-chemical and benthic macroinvertebrates during the dry period (cool and hot seasons) were obtained. The stream classification could be divided into three groups: the reference group (48 stations), the stressed group (42 stations), and the intermediate group (165 stations). Twelve out of 56 metrics have been considered as a core metric for the development of a biological index for quality streams in the Northeast, including Total taxa, EPT taxa, Ephemeroptera taxa, Coleoptera taxa, % EPT, % Chironomidae, % Tolerant individuals, % Intolerant individuals, Beck's index, HBI, Predator taxa, and Clinger taxa. Moreover, this metric set covered the structure and function of organisms including the diversity of species, community structure, tolerance/intolerance measures, functional feeding group, and habit. From the efficacy validation of the biological index, the results of stream assessment corresponded to the classification sites with the physico-chemical characteristics.

**Keywords:** benthic macroinvertebrate index; biological index; multimetric index; stream assessment

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### 1. Introduction

The development of a country has negative effects on streams. As in many other countries in the world, increasing population, economic, urban, agricultural and industrial growth in Thailand are the main causes of changes in water quality. The increased pollution from human activities beyond the carrying capacity of the water resources can contribute to the degradation of water quality. Irrespective of differences in natural character of water sources, the pressures have intensified on tropical streams (Jacobsen and Encalada, 1998).

Water quality assessment can be done by several methods such as physical and chemical methods. In addition, biological methods can also be used for evaluation. It has been found that living organisms in the aquatic environment can be used as an indicator of

the water quality. This can be seen from the wide use of benthic macroinvertebrates for water quality assessment, because of their suitable life history characteristics and diversity (Bonanda *et al.*, 2006; Helson and Williams, 2013) and because the macroinvertebrates cannot live in an unsuitable environment. Therefore, their presence by a change of species composition and community structure is a respond to environment including habitats and biological changed as well as water quality. This finding is one advantage of using macroinvertebrates as indicators of water quality (Flores and Zafaralla, 2012).

A macroinvertebrate community structure is the basis of a water quality monitoring program, which is popular in many European countries and North America. Many countries or states or water authorities have developed indexes for biological assessment of water quality (Li *et al.*, 2010). The index for

biological assessment of water quality is an integrated assessment by monitoring habitat conditions, water quality, and organisms living in the water. The principle is that the summation of quality of both habitat and water can reflect the community structure of the organisms. Some environmental agencies use only benthos to assess water quality conditions (e.g. BioMAP in Ontario and HBI in Wisconsin). This greatly reduces the cost of a monitoring program while maintaining the same power for water quality classification (Ricker, 1934; Hilsenhoff, 1987). In addition, it can be done in several areas, and gives scientific results that are fast, easy to understand, and are obtained in an environment-friendly way (Barbour *et al.*, 1999).

Habitat assessment was used as a tool for the classification and interpretation of animal data, including an indication of habitat quality and stress level of the animal community, by comparing the reference sites with stressed sites, when both sites have similar ecological characteristics. In addition, the metric is biological attributes of the benthic community that indicate ambient water quality conditions (Hughes *et al.*, 2010), and investigators can also use the metric for meaningful indicator attributes in assessing the status of assemblages and communities in response to perturbation.

At present, biological assessment methods have been tested to predict the impacts of human activities on the level of pollution. Currently, there is no ecological index for rapid bioassessment as a standard method to test streams all over Thailand. Recently, Boonsoong *et al.* (2009), Getwongsa *et al.* (2010) and Uttaruk *et al.* (2011) have evaluated water quality using multimetric techniques for stream assessment in northeastern Thailand. They found that the rapid bioassessment of streams can be performed using the multimetric techniques adopted from the United States Environmental Protection Agency (US EPA) by taking the structure of benthic invertebrate data (Barbour *et al.*, 1999). This assessment process is powerful and easy to use.

The former three studies on multimetric index development by using benthic macroinvertebrate assemblages in three headwater streams in northeastern Thailand (Boonsoong *et al.*, 2009; Getwongsa *et al.*, 2010; Uttaruk *et al.*, 2011) were located in the Khorat plateau (Abell *et al.*, 2008) (Fig. 1) and each basin could establish a biological index by itself. Therefore, the present study aims to develop a benthic macroinvertebrate multimetric index by using all data sets collected from the previous studies for headwater stream assessment in northeastern Thailand, in order to

obtain a biological index that can evaluate the whole region and reduce using multiple indices in specific areas. This metric can indicate the ecological stream integrity and it might be possible to supplement the traditional method that government agencies use to monitor water quality. Moreover, it could be applied in other basins of the country or even in neighboring countries.

## 2. Materials and Methods

### 2.1 Dataset

The data of physico-chemical parameters of water quality, habitat assessment, and benthic macroinvertebrates of three major river basins, the Mekong, the Chi and the Mun were collected (Boonsoong *et al.*, 2009; Getwongsa *et al.*, 2010; Uttaruk *et al.*, 2011). Datasets were made from 2004-2007 in the dry period (cool and hot seasons), which represented 255 samplings.

The macroinvertebrates were sampled using the multihabitat approach (Barbour *et al.*, 1999). The sample was collected by using a D-frame dip net (30 cm width, 500  $\mu$ m mesh). A total of 20 kicks were taken from all habitat types over the length of the reach 100 m. All of samples will be composited into a single sample and be preserved in 95% ethanol. In the laboratory, macroinvertebrate samples were subsampled and counted for 300 ( $\pm$  60) individuals (Boonsoong *et al.*, 2009). Benthic macroinvertebrates would identify to the lowest possible taxon mostly in the genus level.

### 2.2 Stream classification

The biological multimetric index is based on a reference condition approach (Barbour *et al.*, 1999). The site classification criteria to identify the quality of streams consist of some physico-chemical characteristics (Table 1).

The sampling sites were classified into three levels of impairment: (1) reference group was sites which must have met all of the listed criteria in Table 1, (2) stressed group was sites which met at least one of all criteria, and (3) a station was not in the two groups above and was classified as an intermediate group (Tetra Tech, 2000a).

The data of benthic macroinvertebrates in each basin was tested in the same ecoregion with ANOSIM (the ANalysis Of SIMilarity) by using PAST (PAleontological STatistics) Version 1.93 (Hammer, 2009).

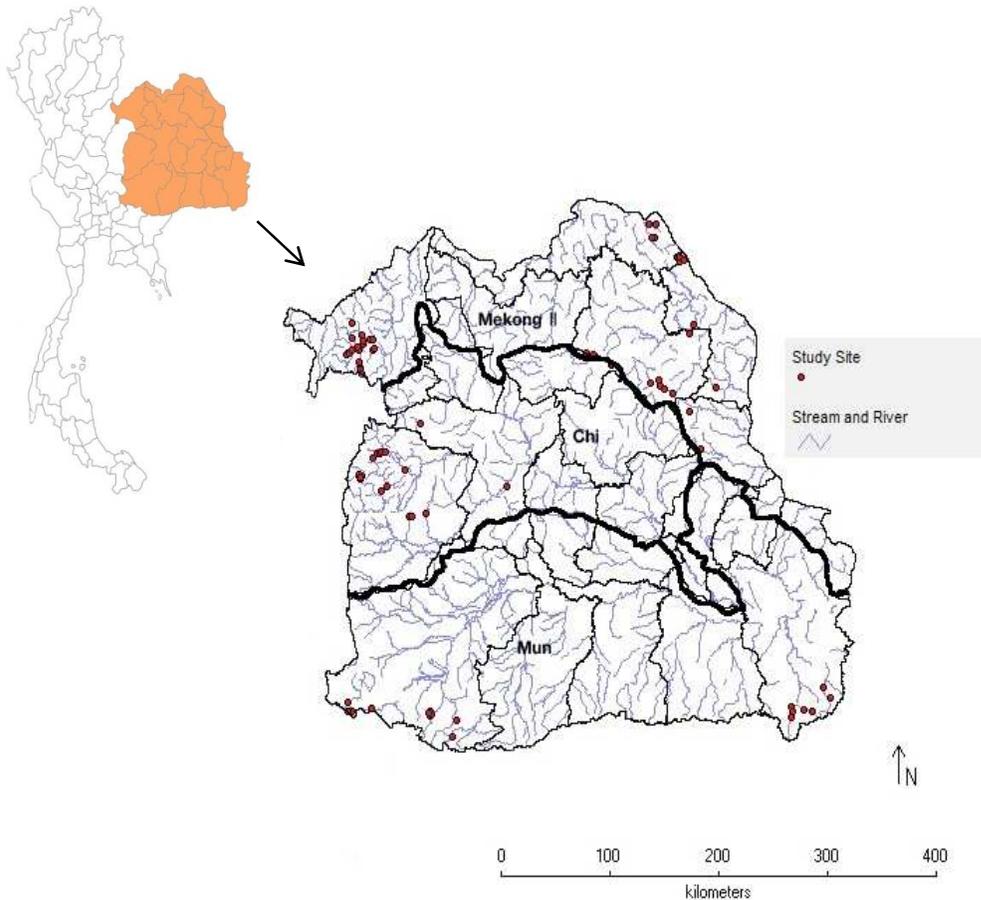


Figure 1. Map showing study sites

### 2.3 Metric selection and calibration

Steps of metric selection followed the procedure of Barbour *et al.* (1996) and Stribling *et al.* (2000). Macroinvertebrate metrics were calculated in EDAS (the Ecological Data Application System), Version 3.3 (Tetra Tech, 2000b) including to characterize taxonomic richness, taxonomic composition, degree of tolerance, functional feeding group and habit group. Sensitivity comparison of the metric values between the reference and stressed sites was made using box and whisker plots. A scoring system was used to evaluate box and whisker plot graphs and a discriminatory ability would intimate candidate metrics.

The metric would measure metric sensitivity and test for redundancy by Discrimination Efficiency (DE).

$$DE = 100 \times (a/b)$$

For metrics that decreased in response to stress,  $a$  is the number of stressed sites scoring below the 25<sup>th</sup> percentile of the reference distribution. In a case where a metric increases in response to stress,  $a$  is the number of stressed sites scoring above the 75<sup>th</sup> percentile of the reference distributions, whereas  $b$  is the total number of stressed sites.

Pearson correlation was used to examine the redundancy between metrics. Any couples of metrics with correlation values more than 0.85 suggested a high relationship between the metrics. This indicated redundancy between metrics and only one metric would be selected for development in the next stage.

The discrete scoring method (Discrete Reference sites used to set expectation, Q1=25<sup>th</sup> percentile reference sites used for expectations) DRQ1 index score was used to convert metrics to the biological index. The categorical scoring system of 1, 3 and 5 points was developed and followed by calculation into a final index (Barbour *et al.*, 1996). The total developed score was divided into five quality classes: excellent, good, fair, poor, and impaired. The biological index value equal to or greater than the 75<sup>th</sup> percentile was classified as being in an excellent condition. If the value was greater than or equal to the 25<sup>th</sup> percentile it was classified as being in a good condition. In contrast, a value less than the 25<sup>th</sup> percentile would be divided two times by the bisection method i.e. fair, poor and impaired conditions (Klemm *et al.*, 2002).

Table 1. Factors used to determine the reference and stressed sites

Factor	Reference sites	Stressed sites	Explanation
Dissolved oxygen (DO) (mg/L)	≥ 6	< 4	Meet the surface water quality national standard
pH	6 – 9	< 4 or > 9	Meet the surface water quality national standard
Electrical conductivity (EC) ( $\mu\text{s}/\text{cm}$ )	< 200	> 1,000	Meet the surface water quality national standard and modified the stress value according
Nitrate ( $\text{NO}_3^-$ -N) (mg/L)	< 5	> 6	Meets the surface water quality national standard
Percent of land use	≤ 20%		Urban land use ≤ 20% of catchment area
Epifaunal substrate score	≥ 11	< 7	The characteristics of habitat structure in the stream. A value ≥ 11 indicated that stream ≥ 40% mix of stable habitat. While, a value < 7 indicated that stream with < 25% stable habitat.
Velocity score	≥ 11	< 7	Pattern of velocity and depth in the sample area. A value ≥ 11 indicated that stream with 3 of the 4 regimes pattern present. While, a value < 7 indicated that stream dominated by 1 velocity/depth regime.
Sediment deposition score	≥ 11	< 7	Sediment-covered the stream bottom. A value ≥ 11 indicated that there are 5-30% of the bottom affected. While, a value < 7 indicated that stream with the bottom sediment more than 50%.
Bank stability score	≥ 11	< 7	Measurement of soil erosion in each bank and then cumulative score together. A value ≥ 11 indicated that 5-30% of bank in reach has erosion. While, a value < 7 indicated that 60-100% of bank has erosion.
Vegetative protection score	≥ 6	< 4	Measurement of the vegetative protection afforded to the stream bank and then cumulative score together. A value ≥ 6 indicated that stream with 50-70% of the stream bank surfaces covered by vegetation. While, a value < 4 indicated that stream with less than 50% of the stream bank surface covered by vegetation.
Percent of total habitat score (score of 200)	≥ 65%	-	A summary of the habitat assessment score according to the US EPA.
Dam present	No	-	The flow of water is not regulated by dam
Point source present	No	-	No point source pollution present
Percent benthic macroinvertebrate taxa	≥ 50 <sup>th</sup>	-	Site which met the 50th percentile of total taxa (28 genera)

(Modified from Pollution Control Department of Thailand, 1997; Barbour *et al.*, 1999; Tetra Tech, 2000a; Jun *et al.*, 2012)

### 3. Results and Discussion

#### 3.1 Stream classification

For the site classification screening criteria, the study sites were divided into three groups composed of the reference group (48 stations), the stressed group (42 stations), and the intermediate group (165 stations). Due to a reference condition approach being used to develop the biological assessment index (Stoddard *et al.*, 2006), the index was considered by the difference of biological metrics between the reference and stressed sites. Any sites classified neither as reference nor stressed sites were classified as intermediate sites. From this study, many intermediate sites were classified, which required clearly distinguishing the metric between the reference and the stressed groups.

The analysis of physico-chemical variables between the reference and stressed sites showed that

the air temperature, water temperature, electrical conductivity, dissolved oxygen, suspended solids, chlorophyll-a, total dissolved solid, and altitude were significantly different ( $p < 0.05$ ). However, the width, water depth, water velocity, pH, nitrate, phosphate, turbidity, and BOD5 between the two conditions were not significantly different ( $p > 0.05$ ).

Two-way ANOSIM indicated that the pattern of benthic macroinvertebrate distribution in each basin was not significantly different ( $R = 0.09$ ,  $p = 0.24$ ) as well as the distribution of macroinvertebrates in each season was not significantly different ( $R = 0.07$ ,  $p = 0.35$ ). Therefore, it is possible to integrate information from all three previous basins into the same ecoregion, due to these three basins being located in the Khorat Plateau (Mekong) ecoregion that covers northeast Thailand and some areas of Lao PDR (Abell *et al.*, 2008).

### 3.2 Metric selection

The study found that the biodiversity of the reference group was greater than the stressed group with 175 and 153 taxa, respectively. The EPT and Coleoptera were common in the reference sites, while Chironomidae was usually found in the stress sites. Fifty-six metrics were calculated in EDAS and then were selected for candidate metrics by using DE between the reference and stressed sites. The analysis of metric selection by box and whisker plots clearly showed the differences between groups. It was found that any metrics that could not separate between the reference and the stressed stations was unable to use for evaluation in that ecosystem (Karr and Chu, 1999). In addition, twenty candidate metrics passed the box and whisker plots test and were selected for evaluation by the distribution of metric raw scores between the reference and stressed sites. In the case of any metrics which did not have a low value and with the DE more than 50%, they could be considered as candidate metrics (Table 2). Therefore, the metric with the lowest score was eliminated due to its inability to rank the score. From this study, clinger taxa were selected into the process of bioindex development although the DE value was 47.60%, as it is a representative metric of the habit group. In all, thirteen metrics were chosen as candidate metrics in order to establish the biological index representing the measure of the five categories.

The results of the redundancy test showed that there were two pairs of metrics with Pearson correlation coefficient greater than 0.85 (Table 3), which were (1) Margalef's index and predator taxa, and (2) EPT taxa and clinger taxa. For the first pair of redundant metrics, the predator taxa were selected for development as an index in the last step, because it was a representative of the functional feeding group. For the second pair of redundant metrics, both of EPT taxa and clinger taxa were selected due to EPT taxa having a high DE value and being a common metric of the benthic macroinvertebrate multimetric index. In addition, the clinger taxa metric was also a representative of the habit group. From the present study, 12 core metrics were considered for the metric selection process, which included total taxa, EPT taxa, Ephemeroptera taxa, Coleoptera taxa, % EPT, % Chironomidae, % tolerant individuals, % intolerant individuals, Beck's index, HBI, predator taxa and clinger taxa. All 12 core metrics comprised five categories of community attributes, taxonomic richness, taxonomic composition, tolerance and intolerance, functional feeding group and habit tendencies, which showed a wide variety

of structural elements and covered the response of benthic macroinvertebrates to all aspects of environmental changes.

The results of this study showed that total taxa, Beck's index and clinger taxa were members of the core metric, which was in agreement with the reports of Boonsoong *et al.* (2009), Getwongsa *et al.* (2010) and Uttaruk *et al.* (2011). Ephemeroptera taxa, % tolerant individuals and % intolerant individuals were the core metrics of the Mekong II basin. In addition, % intolerant individuals metric was the core metric of both the Chi and the Mun basins. Total taxa, EPT taxa, Ephemeroptera taxa, and Coleoptera taxa are members of the taxonomic richness category. Percentage of EPT and % Chironomidae are taxonomic composition measures. The percentage of tolerant individuals, % intolerant individuals, Beck's index and HBI are measures of tolerance and intolerance level category. Predator taxa are functional feeding groups and clinger taxa are measures of habit group. These indices were consistent with the principles of Karr and Chu (1999), who stated that most multimetric biological indices for aquatic systems should consist of 8 to 12 metrics.

### 3.3 Relationship between metric and physico-chemical parameters

Pearson's correlation coefficients have shown that twelve metrics were significantly correlated with physico-chemical parameters ( $p < 0.01$ ). In particular, altitude was the most significant with metric values. The study of Jun *et al.* (2012) also found that altitude was the most associated with the metric score. In addition, Ephemeroptera taxa, Coleoptera taxa, % EPT, and predator taxa had positive relationships with physical parameters, while both % Chironomidae and % tolerant individuals had negative relationships with physical variables. Moreover, Coleoptera taxa had positive relationship with chemical parameters.

### 3.4 Metric calibration and development

The ranking of the core metric score of the reference sites was done by assigning from the percentile of each metric a score of 5, 3 or 1. As shown in Table 4, nine metrics were expected to decrease and three metrics were expected to increase when pollution or disturbance increased.

The percentile of index score as presented in Table 5 shows the range of the multimetric index by using a numeric assessment of 1-5 stream quality classes, which was grouped to impairment levels as excellent, good, fair, poor, and impaired.

Table 2. Candidate metrics with expected response to stress, metric value, Discriminatory Efficiency (DE), and final index

Metric	Expected response	Metric value	DE	Selected to final index
Total taxa	Decrease	32.00	100.00	+
EPT taxa	Decrease	11.00	64.30	+
Ephemeroptera taxa	Decrease	6.00	59.50	+
Plecoptera taxa	Decrease	1.00	57.10	
Coleoptera taxa	Decrease	3.00	59.50	+
Margalef's index	Decrease	3.89	57.10	
% EPT	Decrease	43.34	57.10	+
% Chironomidae	Increase	16.31	57.10	+
% Diptera	Decrease	12.97	19.00	
% Plecoptera	Decrease	0.41	61.90	
% tolerant individuals	Increase	35.63	71.40	+
intolerant taxa	Decrease	2.00	42.90	
% intolerant individuals	Decrease	2.32	57.10	+
% dominant individuals	Increase	40.45	40.50	
Beck's index	Decrease	7.00	66.70	+
Hilsenhoff's Biotic Index (HBI)	Increase	5.85	81.00	+
predator taxa	Decrease	6.00	50.00	+
% borrower	Decrease	9.33	16.70	
clinger taxa	Decrease	9.00	47.60	+*
% clinger	Decrease	34.10	45.20	

\* Selected because of being a representative of the habit category

Table 3. Pearson correlation coefficients of 13 candidate metrics from the reference sites

Metric	Total taxa	EPT taxa <sup>§</sup>	Ephemeroptera taxa	Coleoptera taxa	Margalef's Index <sup>§</sup>	% EPT
Total taxa	1.00					
EPT taxa	0.37**	1.00				
Ephemeroptera taxa	0.19	0.75**	1.00			
Coleoptera taxa	0.35**	0.40**	0.23	1.00		
Margalef's Index	0.33*	0.74**	0.60**	0.68	1.00	
% EPT	-0.26*	0.11	0.28*	0.02	0.16	1.00
% Chironomidae	0.19*	0.03	0.04	0.01	-0.15	-0.30*
% tolerant	-0.16	0.04**	0.04	-0.09	-0.05**	-0.02
% intolerant	0.17	0.51**	0.27*	0.31*	0.62	0.20
Beck's index	0.38**	0.78**	0.46**	0.43	0.71**	0.16
HBI	-0.16	-0.07	0.00	-0.06	-0.17	-0.12
predator taxa <sup>§</sup>	0.25*	0.64**	0.46**	0.63	0.89** <sup>§</sup>	0.13
clinger taxa <sup>§</sup>	0.44**	0.89** <sup>§</sup>	0.56**	0.50**	0.75**	0.19

Metric	% Chironomidae	% tolerant	% intolerant	Beck's index	HBI	predator taxa	clinger taxa
% Chironomidae	1.00						
% tolerant	0.48**	1.00					
% intolerant	-0.23	-0.17	1.00				
Beck's index	-0.15	-0.13	0.78**	1.00			
HBI	0.69**	0.82**	-0.47**	-0.33*	1.00		
predator taxa	-0.08	0.00	0.51**	0.58**	-0.08	1.00	
clinger taxa	-0.06	-0.05	0.53**	0.77**	-0.13	0.69**	1.00

\*\* Correlation is significant at the 0.01 level

\* Correlation is significant at the 0.05 level

§ Redundancy metrics

Table 4. Statistics of frequency distribution by the scoring criteria of the reference sites (min. = minimum, max. = maximum)

Metric	Statistic value of reference sites					Scoring criteria		
	min.	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	max.	5	3	1
Total taxa	28	32	34.5	39	49	≥ 32	31-16	< 16
EPT taxa	6	11	14	19	27	≥ 11	10-6	< 6
Ephemeroptera taxa	3	6	8	9	13	≥ 6	5-3	< 3
Coleoptera taxa	0	3	3	5	9	...	≥ 3	< 3
% EPT	14.8	43.3	60.3	71.0	97.7	≥ 43.3	43.2-21.6	< 21.7
% Chironomidae	0.3	5.1	10.5	16.3	42.4	≤ 16.3	16.2-24.5	> 24.5
% tolerant	0.8	13.8	23.7	35.6	79.4	≤ 35.6	35.5-53.4	> 53.4
% intolerant	0.0	2.3	7.9	18.4	39.4	...	≥ 2.3	< 2.3
Beck's index	4.0	7.0	9.0	14.8	25.0	≥ 7	6.9-3.5	< 3.5
HBI	4.1	4.7	5.4	5.9	6.8	≤ 5.9	5.8-8.9	> 8.9
predator taxa	3	6	7.5	10	17	≥ 6	5-3	< 3
clinger taxa	4	9	13	18	25	≥ 9	8-5	< 5

### 3.5 Verification of multimetric index

Based on the degree of overlap of the score ranges between the reference and stressed sites, the result of box and whisker plot analysis of the benthic macroinvertebrates index revealed that the multimetric index scores between the reference and stressed groups were different, with no overlap of interquartile ranges. Meanwhile, the biological index score of the intermediate group was among the reference and stressed groups (Fig. 2 (A)). These results were consistent with the grouping sites by the standard physical and chemical characteristics according to Table 1. Whereas, the Thai WQI approach could not distinguish between the reference and stressed sites, due to the evaluation score of both reference and stressed sites were overlap (Fig. 2 (B)).

From this study, we can confirm that the benthic macroinvertebrates index approach is effective in assessing the ecology of streams in northeastern Thailand, which was supported by Karr and Chu (1999) who stated that it can be used as a robust tool for measuring stream health and biological conditions of the community. Thus, it may be possible to use this index to assess stream health throughout the whole country, which will be further studied in the future.

### 4. Conclusions

From this study, the stream classification criteria were clearly separated between the reference and stressed sites. The core metric of benthic macroinvertebrates developed was composed of 12 metrics: total taxa, EPT taxa, Ephemeroptera taxa, Coleoptera taxa, % EPT, % Chironomidae, % tolerant individuals, % intolerant individuals, Beck's index, HBI, predator taxa and clinger taxa. All of these 12 metrics represent the measure of five ecology categories: diversity measurement, community measurement, tolerant or sensitivity measurement, functional feeding group and habit group. Biological index score was determined as five conditions: excellent, good, fair, poor and impaired. It was also found that benthic macroinvertebrates index performance results can clearly discriminate the stream condition of the reference sites from the stressed sites. Therefore, it may be a complementary method to the traditional standard approach for assessment of stream ecological integrity. However, this benthic macroinvertebrate multimetric index need more further study to test the performance and the ability to assess the quality of headwater streams in other basins of the country.

Table 5. Ranges of index qualitative scoring

Stream quality class	Percentile of index score	Index score
Excellent	≥75 <sup>th</sup>	54
Good	≥25 <sup>th</sup>	46-53
Fair	<25 <sup>th</sup>	23-45
Poor	-	12-22
Impaired	-	<12

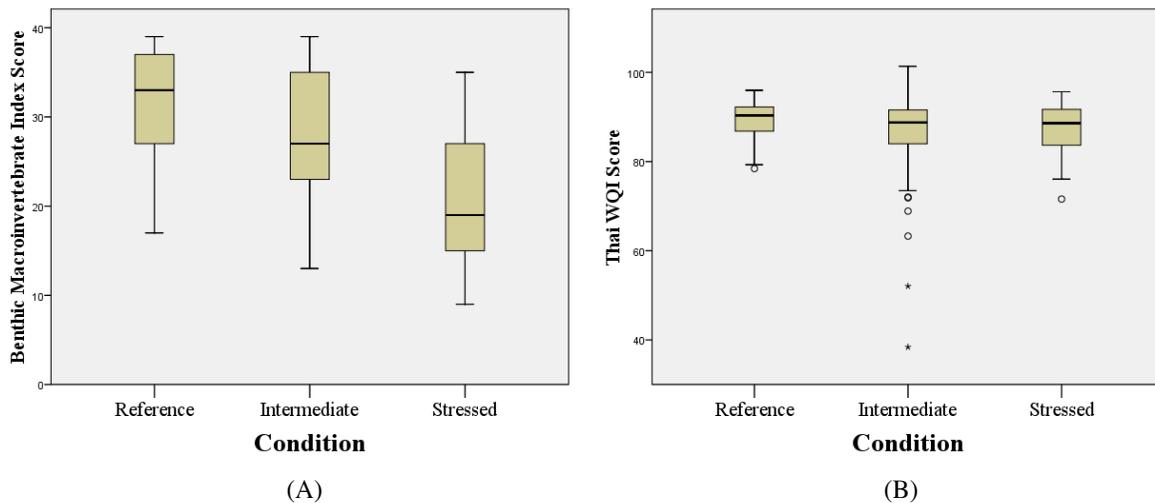


Figure 2. Box and whisker plot graph of the evaluation score (A) the benthic macroinvertebrates index method and (B) Thai WQI method in the reference, intermediate, and stressed groups

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