

Development of biotic index based on rapid bioassessment approaches using benthic macroinvertebrates for Chi and Mun headwater streams, northeast Thailand

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Abstract. The objective of present study is to select high performance metrics for developing a multimetric biotic index to assess headwater stream conditions of the Chi and Mun basins in northeastern Thailand (Khorat Plateau (Mekong) ecoregion) by using benthic macroinvertebrates based on rapid bioassessment protocols (RBPs). Multimetric biotic indices were developed by two methods (decrease and continuous methods). Nine statistically valid metrics were selected from 24 candidate metrics. These nine metrics represent taxa richness, community composition, tolerance/intolerance, functional feeding and habit measure and include the number of total taxa, number of EPTC taxa, Margalef index, Beck's biotic index, Simpson's index, percent intolerance, number of filterer's taxa, number of scraper taxa, and the number of clinger taxa. The results of the biotic index performance demonstrate that the DRQ1 index score (decrease method) produced slightly higher correlation with total habitat score ($r = 0.82$, $p < 0.001$) than did the CAU index score (continuous method, $r = 0.81$, $p < 0.001$). A narrative assessment analysis is recommended when using a multimetric index approach and appears to provide a useful assessment of stream conditions in the Chi and Mun basins.

Key Words: benthic macroinvertebrates, biotic index, rapid bioassessment, multimetric index, headwater streams, Thailand.

Introduction. Thailand is a developing country. Thus, the major sources of water pollution are domestic sewage, industrial wastes, and agricultural wastes. The major impact of water pollution is the degradation of water supply sources effecting the aquatic ecosystem and public health (Boonsoong et al 2010; Pollution Control Department 2010). Currently, most of the criteria for national standards of water quality assessment are based on chemical integrity which cannot reflect the response to multiple stressors to aquatic resources. Only coliform bacteria measurement can be considered a surrogate for biological parameters (Pollution Control Department 1997). The biological index is a more accurate approach to assessing multiple and cumulative stressors to surface waters by evaluating the condition of biological communities (Karr & Chu 1999). The development and use of biological assessment approaches to monitoring water quality have been implemented in many countries in the European Union (European Union Water Framework Directive 2000) as well as in the United States (Barbour & Yoder 2000; Barbour et al 2000). Recently, the development of biological assessment approaches such as the multimetric approach for Thai streams was conducted using benthic macroinvertebrates and Rapid Bioassessment Protocols (RBPs) (Boonsoong 2007).

Results of this research suggest that bioassessment using benthic macroinvertebrates are effective for rivers and streams of Thailand (Boonsoong et al 2009). In order to support the use of this approach as a foundation throughout the country, testing the protocols in other regions is still necessary (Boonsoong et al 2009). The aim of the present study is to develop a biotic index using benthic macroinvertebrates and test the Rapid Bioassessment Protocols for the Chi and Mun headwater streams in Northeastern Thailand.

Materials and Methods

Study area

The study area, the Chi and Mun basins, is located in the Khorat Plateau (Mekong) ecoregion (Abell et al 2008), and covers two-thirds of the area of northeastern Thailand. Most of the land in this region is used for agriculture. The major land uses are the cultivation of rice, sugarcane, cassava, rubber plantations and mixed orchards. Most of the forests are fragmented into small patches. Large area forests are mostly in the protected areas such as national parks and wildlife sanctuary. Generally, the main stresses on aquatic ecological health of this region are from agricultural activities, changes in land use, and waste from urban and industrial areas. Severe natural disturbances occur during rainy season, especially from flooding during the monsoon period. Natural stream condition areas (least or minimal disturbed conditions) are limited and located in protected areas. Sampling stations were chosen base on accessibility, covered both natural and impaired condition. Sampling locations and the distribution of sampling sites are illustrated in Figure 1.

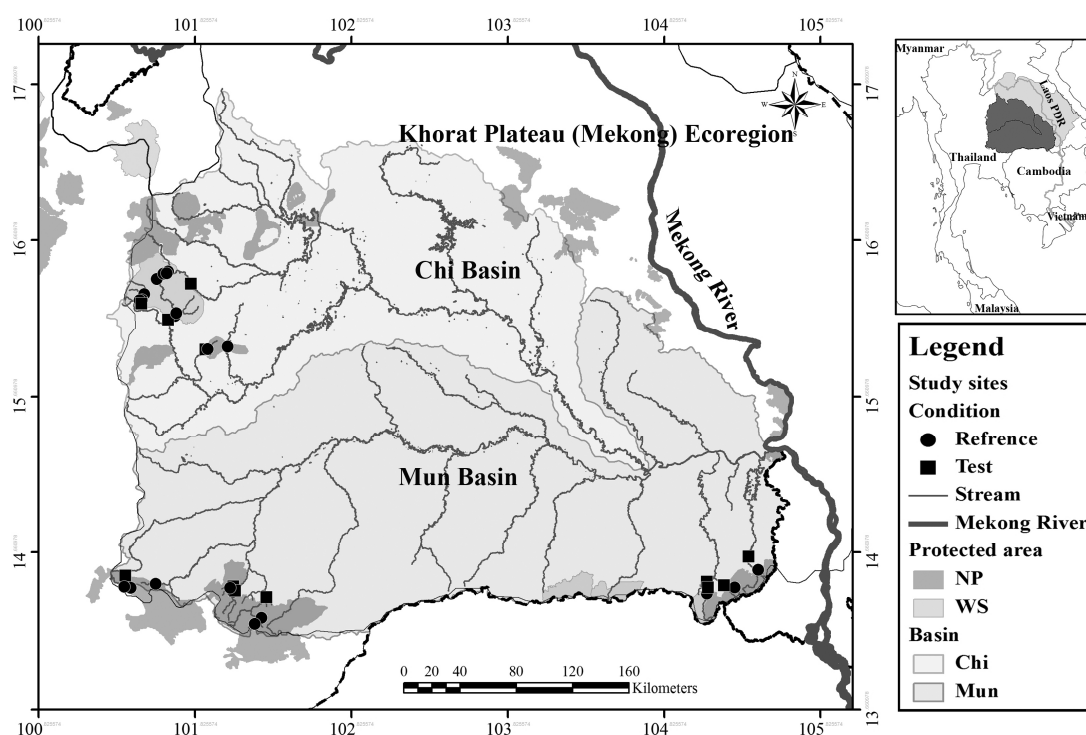


Figure 1. Map showing the sampling locations in headwater streams of the Chi and Mun basins in the Khorat Plateau (Mekong) ecoregion.

Water quality and habitat characteristic assessment

Water samples and habitat characteristics assessment data were collected during hot (April) and cold (November and December) seasons in years 2006 and 2007. Eleven physicochemical parameters were measured including air temperature ($^{\circ}\text{C}$) measured with liquid-in-glass thermometer, dissolve oxygen (mg L^{-1}) with YSI Dissolve Oxygen meter Model 57, pH and water temperature ($^{\circ}\text{C}$) with the sensIonTM 1 Portable pH meter,

conductivity ($\mu\text{S cm}^{-1}$) and total dissolve solid (mg L^{-1}) with Fisher Science method 09-326-2, turbidity (FAU), suspension solids (mg L^{-1}), nitrate ($\text{mg L}^{-1} \text{NO}_3\text{-N}$, ascorbic acid method), and orthophosphate ($\text{mg L}^{-1} \text{PO}_4^{3-}$, cadmium reduction method) measured using the Hach DR/2010 spectrophotometer model 49300-00. Biochemical oxygen demand (BOD_5 , mg L^{-1}) was determined as the difference between initial and 5-day oxygen concentrations in dark bottles after incubation at 20 °C. Chlorophyll *a* ($\mu\text{g L}^{-1}$) was measured with an extracted methanol method (APHA AWWA WPCF 1998). Habitat physical properties of each site were assessed using the format recommended by the USEPA (Barbour et al 1999). Habitat quality was assessed on the sampling reach as the biological sampling. The visual-base habitat assessment technique was evaluated for each parameter (Barbour et al 1999).

Benthic macroinvertebrates sampling and processing

Benthic macroinvertebrates were collected from each site using a multi-habitat approach (Barbour et al 1999; Boonsoong et al 2009) by D-frame dip net (0.3 m wide, 500 μm mesh). A total of 20 kicks were collected proportionately from all major habitat types over the length of reach. Contents of all 20 kicks were composited into a single sample and preserved in 70% ethanol. Benthic macroinvertebrate samples were rinsed in 500 μm mesh sieve and large material was discarded. A suitable number of fixed-count subsampling (300 ± 20 organisms) were collected from each sample following the recommendation of previous study for Thai streams using the RBPs (Boonsoong et al 2009). All organisms from the sorted subsample were identified to the lowest possible taxon, usually genus or species. Identification was base on the reference text "Identification of Freshwater Invertebrates of the Mekong River and Tributaries" (Sangpradub & Boonsoong 2006). The organisms were assigned to an operation taxonomic unit (OUTs).

Data analysis

The Ecological Data Application System (EDAS) (Tetra Tech 2000a) was used as a model for database development (Boonsoong et al 2009). This program is designed to store and analyze ecological data related to bioassessment of surface water and facilitate data analysis, particularly the calculation of biological metrics and indices. Water quality, physical characteristics, habitat assessment and benthic macroinvertebrates data were entered into EDAS 3.3 for data management and analysis. Biological metrics were calculated within EDAS. Benthic Master Taxa and three tables were developed in EDAS that organized tolerance values, functional feeding groups, and habits following Morse et al (Morse et al 1994). Final ID with tolerance value ≤ 3 were considered "intolerant", whereas those with value ≥ 7 were considered "tolerant".

Site classification

To classify sites and establish reference condition (Barbour et al 1999) for this region, *a priori* and *a posteriori* approaches were applied. Macroinvertebrate assemblages were analyzed by non-metric multidimensional scaling (NMDS) based on Brey-Curtis dissimilarity coefficient in PC-ORD ver.5 (McCune & Mefford 2006). This method has been shown to be a robust ordination of species composition and has proven successful for the classification of stream communities (Barbour et al 1996a; Reynoldson et al 1997). Data were transformed by $\log x + 1$ before entering to NMDS analysis.

Index development

The five major steps were involved in the benthic macroinvertebrates multimetric index developing for seasonal biotic index (Barbour et al 1999). Details of process were explained flow these steps.

Metric screening

The concepts of the metric screening process are to identify optimal metrics that measure a predicted response only to anthropogenic disturbances while not influenced by measurement error or natural variability. Overall benthic macroinvertebrates metrics

were selected from the literature of previous research papers (Barbour et al 1996a; Boonsoong et al 2009; Tetra Tech 2000b), and calculated within EDAS. The metrics represent the ecological attributes, including taxonomic richness, community composition, tolerance/intolerance, functional feeding, and habit. All metrics were tested for variability and sensitivity by comparing the value range between reference and test site in each season. Box and whisker plots were used to determine an appropriate suite of metrics that displayed on examination of 25th percentile median, and 75th percentile values of the reference site population for each metric (Barbour et al 1999), then a unit-less score of 0, 1, 2 or 3 were assigned to each metric base on the degree of overlap of value ranges in two sets of population (reference and test sites) (Barbour et al 1996b). Discrimination efficiency (DE) was used for quantitative comparison of discrimination ability for each a metric (Stribling et al 2000; Tetra Tech 2000b). Those metrics which not display box plot differences between reference site and test site and which had discrimination efficiency (DEs) lower than 50% in both seasons were rejected in this step. The remaining metrics were evaluated for their responsiveness to several disturbances such as physical characteristics, chemical characteristics, and habitat quality. Responsiveness was evaluated by using Pearson's correlation. Metrics with large correlation number and p -value ≤ 0.01 were considered significant. The remaining metrics were responsive to disturbances at least one type of disturbance.

Metric selection

Metrics which passed the initial screening steps were evaluated for redundancy by correlating the remaining metrics using a Pearson's correlation coefficient. Metrics with a correlation coefficient (r) > 0.85 were considered highly redundant. Only one metric from a group redundant metric was selected and included in developing the final index.

Metric scoring methods

Two metric scoring methods were used to develop the multimetric index for present study. The first DRQ1 (D= Discrete, R= Reference sites used to set expectation, Q1 = 25th percentile of reference site used for expectation) was used for discrete scoring method and CAU (C= Continuous, A= All sites used to set expectation, U= Upper expectation set (all sites only)) was used for continuous scoring method (Blockson 2003). Scoring process for the first method, each metric was scored by creating a value range from the reference site population whereby a 1, 3 and 5 point categorical scoring system was developed for each metric (Barbour et al 1996a) For the second method, to score metrics, the range of values for each metric was standardized on a 100 point scale, assigning all metrics values a score ranging from 0 (worst) to 100 (best) (Tetra Tech 2000b).

Index Aggregation

To summarize the multimetrics index to single final index the DRQ1 index score (the values score from discrete scoring method of core metrics were summed into single score index) and the CAU index score (the score from continuous method of core metrics) were averaged into a single numerical index value. Six trial index models were then calculated and evaluated to find the best index with the greatest DEs.

Index evaluation

To assess the final index performance, Pearson correlations were used to evaluate the final index between each method and the physicochemical parameters and habitat quality scores. To evaluate the metrics and the final index variation between basin and seasonality we used a 2-way ANOVA test.

Stream Condition Index assessment

Distribution of SCI scores of all reference sites were used to set thresholds between five ordinal ratings of stream condition. Sites equal or greater than the 75th percentile were rated as "very good", and those equal or greater than the 25th percentile were rated as "good". While biotic index with index scores falling below the 25th percentile of reference

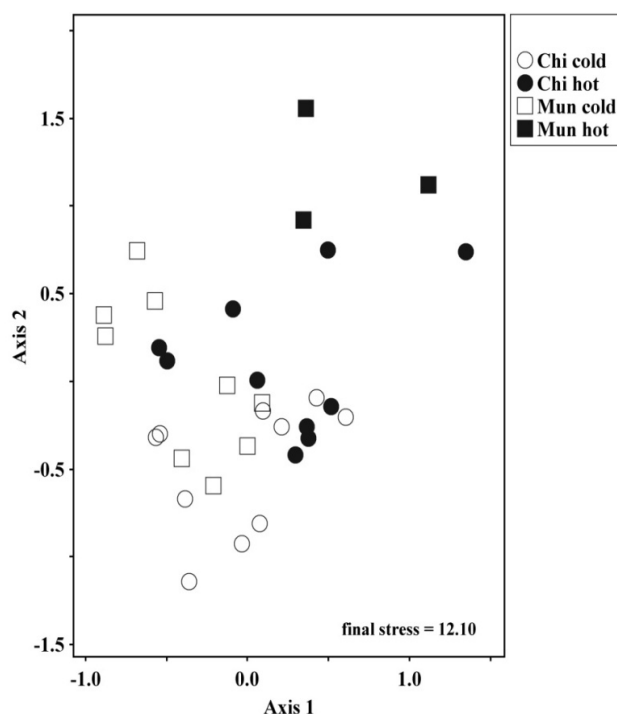
sites population were rated using a bisect method in three ordinal ratings as “fair”, “poor”, and “very poor”.

Results

Site classification

Reference sites were classified base on benthic macroinvertebrates composition. Results from the NMDS ordination (Figure 2) illustrate that reference sites were aggregated in ordinal space. Although some reference sites were spread from the reference group but there was no strong natural variability from spatial factors among reference sites to suggest multiple site classes, Figure 2 illustrates the distribution pattern of the reference sites in the Chi and Mun basins data set from hot and cold season, placed in ordination space with final stress equal to 12.10.

Figure 2. Non-metric multidimensional scaling ordination biplot of the reference sites among basins and collecting seasons based on benthic macroinvertebrates composition.



Index development

Metric screening

Fifty six potential metrics from previous studies for this region were calculated from EDAS and tested for discriminatory ability of anthropogenic disturbance. Box plots revealed that thirty two metrics were not different between reference sites and test sites. The results of the discrimination efficiency (DEs) of these metrics are less than 50% in both seasons. Twenty four metrics in five categories were defined as candidate metrics (Table 1). Pearson correlation coefficients revealed that all of these metrics are highly correlating ($p < 0.001$) with physicochemical parameters or habitat quality score for at least one parameter. Metrics with low value range or many zero values were classified as low performance metrics and were omitted from the redundancy test (Table 1).

Metric selection

Twelve of twenty four core metrics were entering into the redundancy test. Pearson correlation coefficients revealed strong correlations ($r \geq 0.85$) between total taxa and Margalef index and ETO taxa, and that ETO taxa and EPTC taxa. The most robust metrics (total taxa and EPTC) of taxonomic richness measures were selected for the final index development. Even though the Margalef index was highly correlated with total taxa, it should not necessarily be excluded, because it is the only metric of composition category. In addition, dominant taxon (one taxon) was highly correlated with Simpson's index (0.94) (Table 2). The Simpson's index was considered the most robust of dominant taxon (one taxon) and was retained for final index development. Consequently, ETO taxa, and Dominant taxon (one taxon) were eliminated from the final index development (Table1).

Metrics scoring

The nine remaining metrics, including the number of total taxa, number of EPTC taxa, Margalef index, Beck's biotic index, Simpson's index, percent intolerance, number of filterers taxa, number of scraper taxa, and number of clinger taxa were transformed to unit-less scores using two methods (DRQ1 and CAU) for hot and cold seasons. For the DRQ1 method, categories scoring range were developed base on original data.

Categories scoring range and the descriptive statistic for all core metrics are shown for cold season in Table 3 and for hot season in Table 4. The CAU method was used only for the 95th or 5th percentile (depending on the metrics) standard value determined from the combined set of all samples. Individual metrics in high quality streams may have received scores higher than 100, but a maximum metrics score of 100 was used.

Table 1

Candidate metrics with expected response to stress, discrimination efficiency, and reason for including or excluding metric to final index

Category and metric	Expected response	Discrimination Efficiency		Selected to final index	Reason for including or excluding metric to final index
		Cold	Hot		
Taxonomic Richness					
Total taxa	Decrease	100	77	x	Good DE
Diptera taxa	Decrease	58	39		Low DE
Coleoptera taxa	Decrease	33	62		Low DE
ETO taxa	Decrease	100	54		85% correlated with Total taxa 86% correlated with EPTC taxa
EPTC taxa	Decrease	83	85	x	Good DE
Taxa Composition					
Margalef Diversity Index	Decrease	100	77		99% correlated with Total taxa
% Odonata	Decrease	50	85		Low performance
Tolerance/Intolerance					
Beck's Biotic Index	Decrease	67	92	x	Good DE
Simpson's Index	Increase	75	77	x	Good DE
% Dominant taxon	Increase	75	77		94% correlated with Simpson's Index
Hisenhof's Biotic Index	Increase	17	54		Low DE
% Intolerance	Decrease	83	77	x	Good DE
Intolerant taxa	Decrease	67	100	x	Good DE
Feeding measures					
% Collectors	I Increase	50	46		Low performance
Collectors taxa	Decrease	50	0		Low performance
Filterers taxa	Variable	75	77	x	Good DE
Predators taxa	Decrease	67	62		Low range
% Scrapers	Decrease	50	69		Low performance
Scrapers taxa	Decrease	67	85	x	Good DE
% Shredders	Decrease	92	69		Low performance
Shredders taxa	Decrease	75	39		Low range
Habit measures					
Clingers taxa	Decrease	75	77	x	Good DE
% Spawlers	Decrease	67	23		Low performance
Spawlers taxa	Decrease	92	15		Low performance

Table 2

Pearson correlation matrix of benthic macroinvertebrates metrics in reference site (n=32)

Metric	Total taxa	EPTC taxa	ETO taxa	Margalef Index	Beck's Biotic Index	Simpson's Index
EPTC taxa	0.84**					
ETO taxa §	0.85**	0.86**				
Margalef Index §	0.99**	0.84**	0.83**			
Beck's Biotic Index	0.62**	0.74**	0.51**	0.61**		
Simpson's Index	-0.60**	-0.57**	-0.57**	-0.59**	-0.46**	
Dominant taxon (one taxon) §	-0.46**	-0.46**	-0.45*	-0.45*	-0.45**	0.94**
% Intolerant	-0.04	-0.04	-0.08	-0.05	0.22	-0.17
Intolerant taxa	0.46**	0.52**	0.26	0.47**	0.89**	-0.36*
Filterer taxa	0.63**	0.58**	0.66**	0.60**	0.42*	-0.40*
Scraper taxa	0.02	0.07	-0.09	0.04	-0.06	-0.01
Clinger taxa	0.75**	0.84**	0.82**	0.73**	0.68**	-0.56**
Metric	Dominant taxon	% Intolerant	Intolerant taxa	Filterer taxa	Scraper taxa	
% Intolerant	-0.27					
Intolerant taxa	-0.383*	0.25				
Filterer taxa	-0.27	-0.02	0.24			
Scraper taxa	-0.01	-0.14	0.01	-0.36*		
Clinger taxa	-0.45*	-0.07	0.43*	0.73**	-0.16	

Marked Correlation are significant * $p < 0.05$, ** $p < 0.01$

§ Redundancy metrics

Index aggregation

Core metrics scored values were entered in each index trial model base on each method. The results of the discrimination efficiency of each model in different scoring methods for each season are shown in Table 5. The results showed that the index trial model I had the strongest DEs for both the DRQ1 and CAU indices and for both seasons. Nine core metrics were used to develop the final index for model I. The range of index scores of the reference sites was compared with the range of index scores from the test sites using mean values and displayed by box and whisker plots. The box plots supported the ability of final index discrimination (Figure 3).

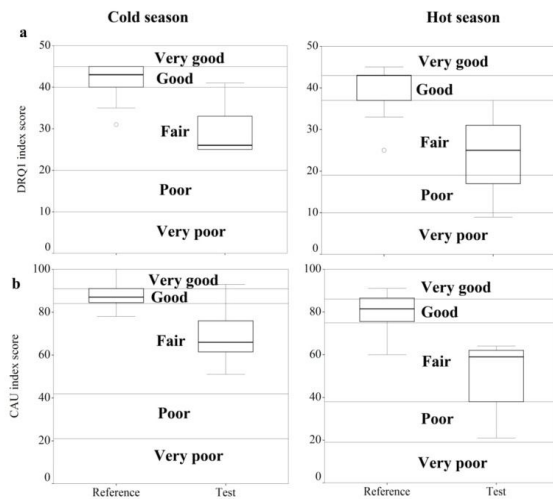


Figure 3. Box plot comparing the index score (a) DRQ1 and (b) CAU between reference and test sites in the two index periods with 5 narrative assessment categories.

Table 3
Descriptive statistic and score for core metrics for the cold season index period DRQ1 scoring method in Chi and Mun headwater streams. Data combined for years 2006 and 2007 (n=20).

Metric	Descriptive Statistic					Categories scoring range		
	Min.	25	Median	75	Max.	5	3	1
No. of Total taxa	30	34	36.5	39	49	≥ 34	17-33	<17
No. of EPTC taxa	18	20	23	26	33	≥ 20	10-19	<10
Margalef Index	5	6	6	7	8	≥ 6	3-5	<3
Beck's Biotic Index	9	9.25	12	15.75	19	≥ 9.25	4.63-9.24	<4.63
Simpson's Index	0.05	0.07	0.10	0.13	0.15	≤ 0.13	0.14-0.26	>0.26
% Intolerant	3.91	10.78	13.38	16.64	28.65	≥ 10.78	5.39-10.77	<5.39
No. of Filterers taxa	5	5.25	7	8	9	≥ 5	3-4	<3
No. of Scraper taxa	2	4	5	5	6	≥ 4	2-3	<2
No. of Clinger taxa	16	17	19	21	23	≥ 17	9-6	<6

Table 4
Descriptive statistic and score for core metrics for the hot season index period of DRQ1 scoring method in Chi and Mun headwater streams. Data combined for years 2006 and 2007 (n=12).

Metric	Descriptive Statistic					Categories scoring range		
	Min.	25	Median	75	Max.	5	3	1
No. of Total taxa	27	28	33	36	37	≥ 28	14-27	<14
No. of EPTC taxa	14	16	21	24	27	≥ 16	8-15	<8
Margalef Index	5	4.7	6	5.98	6	≥ 4.7	2.4-4.6	<2.4
Beck's Biotic Index	6	7.25	10.5	12.75	16	≥ 7.25	3.63-7.24	<3.63
Simpson's Index	0.06	0.09	0.11	0.13	0.22	≤ 0.13	0.14-0.26	>0.26
% Intolerance	5.81	7.02	10.36	24.01	28.97	≤ 7.02	3.51-7.01	<3.51
No. of Filterers taxa	1	3.25	4	5	6	≤ 3	2-3	<2
No. of Scraper taxa	4	5	5.5	6.75	9	≤ 5	3-4	<3
No. of Clinger taxa	9	11.5	15.5	19	20	≤ 12	6-11	<6

Index evaluation

The responsiveness of the final index score to anthropogenic stress was evaluated. The results of the Pearson correlation analysis revealed that index scores were highly correlated with total habitat score for both the DRQ1 ($r=0.82$, $p<0.001$) and the CAU ($r=0.81$, $p<0.001$) methods (Figure 4). Furthermore, there was high correlation between the DRQ1 and CAU index score ($r=0.92$, $p<0.001$). Both index scores were negatively correlated with increasing water temperature ($r>-0.51$, $p<0.001$), turbidity ($r>-0.34$, $p<0.05$), biochemical oxygen demand (BOD, $r>-0.46$, $p<0.001$) and chlorophyll a ($r>-0.41$, $p<0.001$). The result of the ANOVAs of metrics and index from the reference site data showed significant differences between seasonality factors for 8 of the 9 metrics and the CAU index score ($p<0.05$). Percent intolerance for metric and DRQ1 index score were not significantly different. Each metric and index usually had higher mean values of cold than hot season. A significant difference of metrics and index between basins was found only for the CAU index score ($p<0.05$). Mean values of each metric and index were usually higher in the Chi headwater than in the Mun headwater streams. Finally, a test of interactions between basin and season factors was not significantly different in all cases. F-values for main factors and interactions with the level of significance are showed in Table 6.

Table 5

Six trial index models were developed by aggregation of core metrics to single final index and evaluated with discrimination efficiency (DEs).

Index trial model		I	II	III	IV	V	VI
Core metrics							
Total taxa		x	x	x	x	x	x
EPTC taxa		x	x	x	x	x	x
Margalef Index		x	x	x	x	x	x
Beck's Biotic Index		x	x	x	x	x	x
Simpson's Index		x		x	x		
% Intolerant		x	x			x	x
Filterers taxa		x	x	x	x	x	
Scrapers taxa		x	x	x			x
Clingers taxa		x	x	x	x	x	
DRQ1 method	DE (Cold season)	92	83	92	92	83	83
	DE (Hot season)	100	100	100	92	100	100
CAU method	DE (Cold season)	92	92	92	92	92	83
	DE (Hot season)	100	100	92	100	100	100

Stream condition index and assessment

The five categories of narrative assessment were divided based on the index value ranges among reference site populations. Table 7 shows the proportion of site ($n=57$) in each assessment method (DRQ1/CAU) and by seasonality (hot/cold) by the five narrative assessment criteria ("very good", "good", "fair", "poor", "very poor"). The results of the assessment (Figure 5) show that for the reference site populations each method showed similar class results: DRQ1 - 53%, 28%, 19% and CAU - 56%, 22%, 22% as "very good", "good" and "fair". However the results of the assessment for the test sites show some variability between the two methods: DRQ1 - "good" = 4%, "fair" = 76%, "poor" = 12% and "very poor" = 4%; CAU - "very good" = 4%, "good" = 4%, "fair" = 80%, "poor" = 16%.

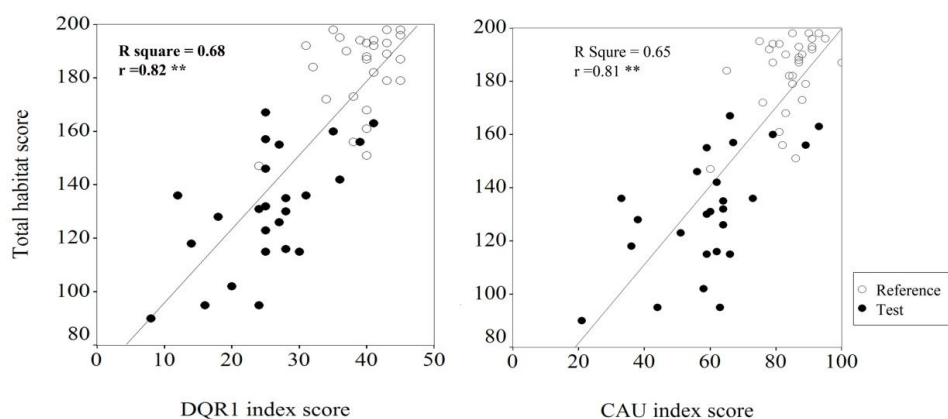


Figure 4. Scatter plots between DRQ1, CAU index scores and Total habitat score in reference and test sites.

Table 6
F-values for main factors and interactions in ANOVA of benthic macroinvertebrates metrics and final index scores calculated from data for reference sample sites (n=32)

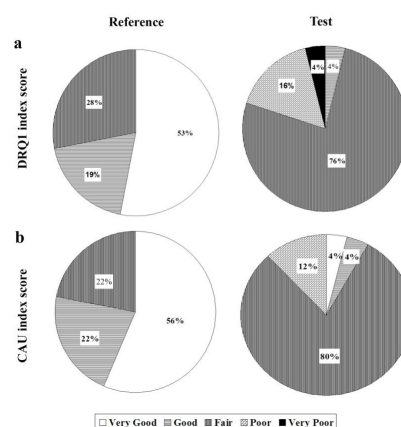
Metric/Index	Factor or Interaction		
	Basin	Season	Basin x Season
Total taxa	0.35 ns	8.27 **	0.35 ns
EPTC taxa	1.65 ns	7.57 **	1.65 ns
Margalef Index	0.65 ns	7.04 *	0.35 ns
Beck's Biotic Index	1.58 ns	5.99 *	0.61 ns
Simpson's Index	3.37 ns	4.49 *	1.86 ns
% Intolerant	0.29 ns	0.03 ns	0.45 ns
Filterers taxa	3.72 ns	31.86 ***	0.03 ns
Scrapers taxa	2.17 ns	6.22 **	0.01 ns
Clingers taxa	3.39 ns	18.06 ***	1.78 ns
DRQ1	2.13 ns	2.48 ns	2.25 ns
CAU	5.87*	16.17***	2.74 ns

ns = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Discussion

Few publications about biotic index development in Thailand have been published (Boonsoong et al 2009; Silalom et al 2010; Thorne & Williams 1997). The biotic indicator development of multimetric approach using benthic macroinvertebrates for Thai streams has only one publication (Boonsoong et al 2009) from the Lower Lancang (Mekong) ecoregion (Abell et al 2008).

Figure 5. Pie diagrams illustrating the percent of narrative assessment of reference and test sites for DRQ1 and CAU methods.



In the present study, biotic indices were developed for the Chi and Mun headwater streams, located in Khorat Plateau (Mekong) ecoregion. This ecoregion covers northeast Thailand and part of Laos PDR (Abell et al 2008). Site classifications using reference site population base on NMDS showed similar patterns with the previous study in Thailand (Boonsoong et al 2009). High variation occurs between the hot and cold season. However, there is not sufficient evidence to suggest a separation of groups based on spatial variation. Most variation of benthic macroinvertebrates assemblages in the reference sites of this study is due mainly to seasonal variation. Previous studies indicate the important role of seasonal variation on stream and surrounding habitat stability and habitat heterogeneity, this could be dominant factor determining macroinvertebrates communities' stability (Poff & Ward 1990; Death 1995; Beisel et al 2000).

Metric evaluation and calibration

Several potential metrics were calculated and selected from EDAS to evaluate anthropogenic disturbance ability for Thai streams based on benthic macroinvertebrates assemblage using current taxonomy (Sangpradub & Boonsoong 2006) and ecological information (Boonsoong et al 2009). In the present study, nine core metrics including total taxa, EPTC taxa, Margalef index, Beck's biotic index, Simpson's index, percent intolerant, filterers taxa, scrapers taxa, and clingers taxa were selected from 24 candidate metrics. The main reason for selection of these metrics is the degree of overlap between the reference and the test site populations shown in the box and whisker plot and discrimination efficiency (DEs) analysis. While the Margalef index was shown to be highly correlated with total taxa from the redundancy test, both of them were included in the final index development, because the consideration of ecological meaning has more importance than statistic evaluation (Karr & Chu 1999). The core metrics which represent the ecological characteristics for this study include species richness, composition, tolerance and trophic structure. The core metrics should be represented divers aspects of structure, composition and individual health as recommended (Barbour et al 1995). Most multimetric biological indices for aquatic systems comprise 8 to 12 metrics (Karr & Chu 1999). Two taxa richness matrices (total taxa and EPTC taxa) were retained and used to develop the final index. Four of nine core metrics which were included in the final index for the study by (Boonsoong et al 2009), namely the number of diptera taxa, percent Plecoptera, percent tolerant and number of shredders taxa were not included in this study. While the Margalef index was strongly correlated with the total taxa, it was the only metrics used which represented the composition measures category. Consequently, it was included in the final index. Typically, Hilsenhoff biotic index (HBI) is very useful in discriminating between higher and lower water quality sites. It was not a clear discriminator, however between the reference and test sites in this study as shown in a previous study (Boonsoong et al 2009). HBI is generally calculated by summing the product of proportion of individuals of each taxon in a sample by its assigned pollution tolerance value (Blocksom & Winters 2006). Also, to refine the HBI performance for this ecoregion, the tolerance values for each taxon may require further refinement or development (Blocksom & Winters 2006). The three metrics in final index, representing tolerant and intolerant measures are Beck's biotic index, Simpson index and percent intolerant. Some studies have suggested that including functional feeding group in biotic assessments reflect the fundamental differences in trophic patterns and nutrient sources among reaches (Hannaford & Resh 1995; Kerans & Karr 1994). In the present study, the Filtering taxa metric was used to represent functional feeding measures. In one study, collector-filterer richness was included in MBII for the Mid-Atlantic Highlands Region (MAHR) and this was shown to detect impairments related to increased sedimentation (Klemm et al 2003). Using the percent filterers was successfully in an assessment for developing the Florida Stream Condition Index (SCI) for wadeable streams (Barbour et al 1996a). The last two metrics included in the final index represented habit measures and were the scrapers taxa and the clinger taxa.

Table 7

Definitions of narrative assessment using index value base on final index model I

Narrative assessment	Percentile of reference index value	DRQ1 index score		CAU index score	
		Cold	Hot	Cold	Hot
Very Good	≥75	45	43	91	86
Good	≥25	40-44	38-42	84-90	75-85
Fair	<25	20-39	19-37	42-83	38-74
Poor	-	10-20	10-18	21-41	19-36
Very Poor	-	<10	<10	<21	<19

Index development and evaluation

The multimeric indices were developed with two different methods, the DRQ1 method (discrete scoring method) (Barbour et al 1999) and the CAU method (continuous method) (Tetra Tech 2000b). The initial assessment of the final index indicates a strong separation between reference and test sites. The index scores of the DRQ1 method and the CAU method performed well and show similar results to the previous Thai stream study (Boonsoong et al 2009). In the evaluation of the final index responsiveness, the CAU index score shows a relatively higher correlation with human disturbance than the DRQ1 index score. However, the DRQ1 index score was relatively more consistent than the CAU index score. Fore (2003) stated that "A highly variable indicator must show a large change in value before the change is statistically significant and lack of sensitivity translates into an inability to sound an alarm that will protect resources from degradation". The ANOVA results for the reference site populations demonstrate that variation within the metrics and indices is a result of seasonal changes (cold versus hot season).

Stream Condition assessment

The result of narrative assessment using the final index revealed that the proportion of "very good" and "good" conditions was high for the reference site groups. "Fair" and "poor" conditions dominated the test site groups for both index development methods (DRQ1 and CAU). The results show that both final indices responded to a variety of stressors affecting stream conditions in this region of Thailand.

Conclusion. The result from the present study support that the development of a biotic index using the multimetric approach is a good technique for rapid bioassessment protocols, assessing the aquatic ecological health for the Chi and Mun headwater in northeastern, Thailand. The result of the stream condition assessment using a biotic index demonstrate that multimetric index can alert people and national agencies to poor and unhealthy stream conditions in Chi and Mun headwaters. In developing a multimetric index for other ecoregions, some of the metrics may require changes in the response levels of the disturbance gradient. In order to implement a nationwide stream health assessment program using biotic indices and rapid bioassessment protocols, refinement and development of the specific biotic indices are needed for each ecoregion of the country. The development of biotic indices is dependent on reference condition criteria within each ecoregion. This work is a prerequisite for a nationwide stream health assessment tool.

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