Development of biotic index to assess the Phong and Cheon rivers' healths based on benthic macroinvertebrates in Northeastern Thailand

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Abstract. Large river bioassessment approaches are poorly reported, especially in Thailand. The aim of this research is to develop appropriate biotic candidates for the rapid bioassessment of large rivers. Benthic macroinvertebrates, some physico-chemical parameter of water quality and habitat assessment data were collected from 13 sampling sites along the Phong and Cheon Rivers during cool, hot and rainy seasons of 2012-2013. A macroinvertebrate sampling method was adopted from the Large River Bioassessment Protocol (LR-BP). At each site, there were a total of twelve transects within the total length of sampling reach of 500 m. At each transect, macroinvertebrates were collected over 6 sweeps using a D-frame dip net. Fourteen physico-chemical parameters of water quality were also measured. The total habitat score in the reference sites was higher than that of the tested sites in all seasons. Electroconductivity (EC), total dissolved solids (TDS), turbidity, suspended solids (SS) and nitrate-nitrogen were significantly different between the reference and test sites ($p < 0.05$). A biotic index comprising eight core metrics selected from 45 metrics tested was developed. The number of total taxa, the number of ETO taxa, the number of Coleoptera taxa, percentage of Trichoptera, Margalef's Index, Beck's Biotic Index, percentage of filterers and percentage of burrowers were calibrated for the final index. The water quality classes in the Phong and Cheon Rivers were categorized into excellent, good, moderate and poor based on the DRQ1 and CAU scoring methods. In addition, these results suggest that using DRQ1 and CAU index scores are more suitable than the use of the early multimetric in Thailand, Ghana and Brazil for assessing the Phong and Cheon Rivers' healths.
Key Words: bioassessment, biotic index, multimetric index, non-wadeable river.

Introduction. Today, the health of rivers has been dramatically changed by anthropogenic disturbances such as agricultural activities, industry, urbanization and recreation. Bioassessment approaches are a broadly applicable indicator used for evaluating the conditions of streams and rivers. These include such indices as the Index of Biotic Integrity (IBIs). The IBI was developed to classify stream impairment using fish assemblages (Kerans & Karr 1994). Many biomonitoring programs have incorporated multimetric indices, which are commonly used for assessing river health throughout the world (Thorne & Williams 1997; Mustow 2002; Flotemersch et al 2006). Monitoring indicators including benthic macroinvertebrates, are often used to reflect the overall community patterns of stream or river conditions (Resh & Jackson 1993; Morse et al 2007; Stoian et al 2009; Dumbravă-Dodoacă & Petrovici 2010). For the Rapid Bioassessment Protocol (RBP), fish, benthic macroinvertebrate and periphyton assemblages are used to develop a multimetric index (Barbour et al 1999). In the USA, the multimetrics were investigated in the St. Crox, Wisconsin, Minnesota, Illinois, Wabash and Scioto Rivers (Blocksom & Johnson 2009) and in Wisconsin nonwadeable Rivers (Weigel & Dimick 2011). Moreover, multimetrics were investigated in the rivers and lakes in Flanders, Belgium (Gabriels et al 2010) and were also studied in the Cabe River in Spain (Cuadrado et al 2014). Many studies have been developed for biometrics in large rivers. The multimetrics were studied in the Liao River, the Xiangxi River, the Taihu basin and the Huntai River in China (Meng et al 2009; Li et al 2010; Huang et al 2015; Li et al

2016). Aazami et al (2015) studied multimetrics in the Tajan River in Iran. In addition, Nguyen et al (2014) developed the multimetric in the Cau River of Vietnam. In Thailand, Boonsoong et al (2009), Getwongsa et al (2010), and Uttaruk et al (2011) have developed rapid bioassessment methods for Thai streams by adopting the US EPA protocols. In contrast, Phaphong & Sangpradub (2012) developed a biotic index using benthic macroinvertebrates as a bioassessment tool for wetlands. However, a practical method of rapid bioassessment in large rivers which is appropriate for assessment, has not yet been developed in Thailand. Therefore, this study aims to fill this gap by focusing on biotic index for rapid bioassessment to assess the Phong and Cheon Rivers' healths.

Material and Method

Study area and site selection. The study area is located in Northeast Thailand, and the area lies between 16°22'-16°46'N and 102°02'-102°56' E with an altitude ranging from 149 to 264 m above sea level. Field sampling was seasonally conducted during the cool (February), hot (April) and rainy (July and August) seasons of 2012 to 2013. Thirteen sampling sites were established along the downstream parts of the Phong and Cheon Rivers. The Phong River is 230 km in length with a catchment area of 2,142 km^2, which is divided into upstream and downstream by the Ubolratana reservoir. This study selected downstream of the Ubolratana reservoir which features a large river. The river reaches in these sampling sites were separated into the upper reaches of the weir (between Ubolratana reservoir and Nongwai weir, PO01 to PO04), which they are mostly agricultural and residential areas and the lower reaches of the weir (between Nongwai weir and Mahasarakham weir, PO05 to PO09) that are intensive agricultural, residential and industrial areas. The Cheon River is 180 km in length with a catchment area of 1,483 km^2. The Cheon River sites consist of CH01 which is mainly for agriculture, CH02 is surrounded by intensive agricultural areas including corn fields, cassava fields and sugarcane fields, CH03 is surrounded by residential and agricultural areas and CH04 is surrounded by fishing and agricultural areas. They are mostly agricultural, residential areas and aquaculture (Figure 1).

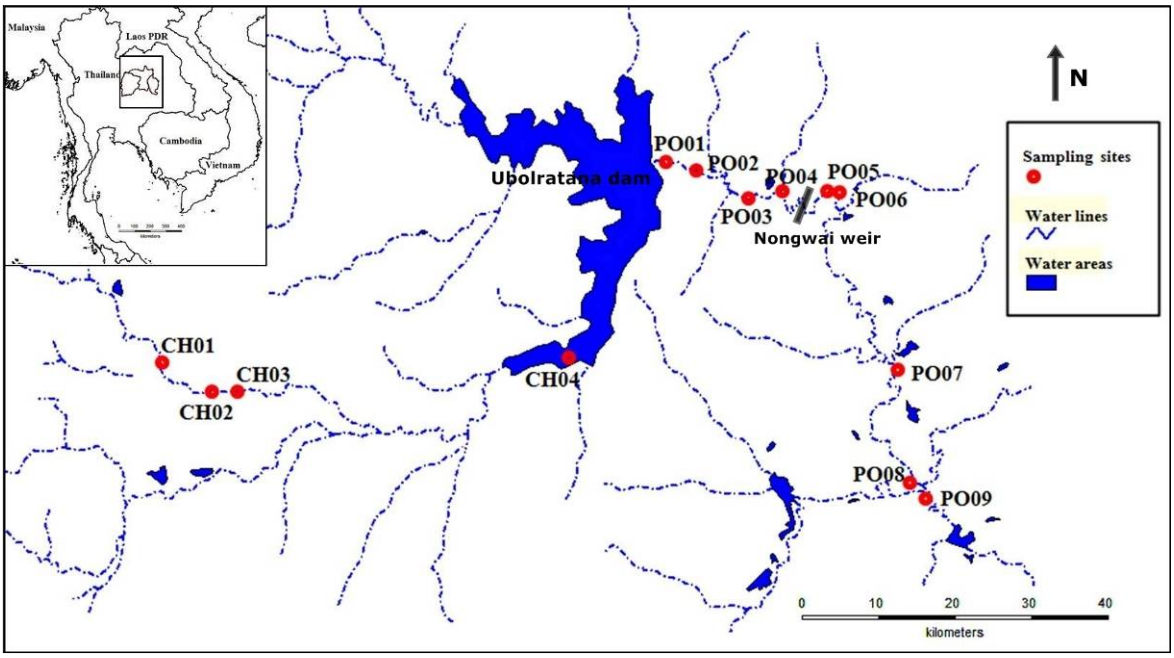

Figure 1. Map showing locations of sampling sites along the Phong and Cheon Rivers.

Reference sites were established in areas that had best attainable conditions (BAC) as possible. BAC sites would be places where land use impacts sites less (Stoddard et al 2006). Physico-chemical parameter of water quality and ecological data were used as the main criteria for selecting the reference sites. They include dissolved oxygen ≥ 4 mg L^{-1},

Biochemical oxygen demand (BOD_5) ≤ 2 mg L^{-1}, pH 5-9, nitrate-nitrogen < 5 mg L^{-1}, ammonia-nitrogen < 0.5 mg L^{-1} (PCD 2004), electroconductivity < 300 µs cm^{-1}, turbidity < 100 FAU and suspended solids < 100 mg L^{-1} (these values follow monitoring of surface water quality during the last five years). The assessment of physical habitat for the reference sites includes riparian width ≥ 10 m, bottom deposition ≥ 50%, bank stability ≥ 60 % and total habitat scores ≥ 56 (Wilhelm et al 2005). The reference sites must satisfy all these criteria; otherwise, they are classified as test sites. This is because they have been affected by a variety of anthropogenic disturbances.

Physico-chemical parameters of water quality and habitat assessment. Water samples were taken in advance to collect benthic macroinvertebrates from each sampling site. *In situ* measurements included depth (m), velocity (m s^{-1}) measured using a flow velocity indicator (Gurley Percision Instruments, Model 1100), water temperature (ºC) and dissolved oxygen (DO, mg L^{-1}) measured by YSI Dissolved Oxygen meter Model 57. Biochemical oxygen demand (BOD_5, mg L^{-1}) was determined to compare the difference between initial and 5-day oxygen concentrations in dark bottles, after incubation at 20ºC. pH, electroconductivity (µs cm^{-1}) and total dissolved solids (TDS, ppm) were tested with pH/EC/TDS meter model HI 98129. Turbidity (FAU), suspended solids (mg L^{-1}), nitrate-nitrogen (mg L^{-1} NO_3^-N, ascorbic acid method), orthophosphate (mg L^{-1} PO^{3-}_4, cadmium reduction method) and ammonia-nitrogen (mg L^{-1} NH_3-N, the Nessler method) were measured using the Hach DR/2010 spectrophotometer model 49300-00. Chlorophyll a (µg L^{-1}) was measured with an extracted-methanol method (APHA AWWA WPCF 1998). The visual-based habitat assessment was evaluated following a non-wadeable habitat index (NWHI) at each site such as riparian width, large woody debris, aquatic vegetation, bottom deposition, bank stability, thalweg substrate and off-channel habitat. NWHI scores ranged from 0-100 points (Wilhelm et al 2005).

Benthic macroinvertebrate sampling. Benthic macroinvertebrate sampling followed the Large River Bioassessment Protocol (LR-BP) (Flotemersch et al 2006) and was performed using a multi-habitat approach within each 500 m reach length. At each site, we sampled a total of six transects from each bank. At each transect, the macroinvertebrates were collected for six sweeps using a D-frame dip net (0.3 m wide, 450 µm mesh). All samples from both bank sides were combined into a single sample and preserved in the field with 70% ethanol. In the laboratory, specimens were rinsed in 500 µm mesh sieves and large organic materials were removed. All organisms from the sorted sample were identified to the lowest possible taxonomic taxon, usually genus except Oligochaeta (Class) and Diptera (Subfamily) based on Morse et al (1994), Yule & Yong (2004) and Sangpradub & Boonsoong (2006). Those specimens were assigned to operational taxonomic units (OTUs).

Data analysis. Benthic macroinvertebrate assemblage data were analyzed using clustering and ordination by non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarly distance in PC-ORD ver. 5 (McCune & Mefford 2006). Independent-sample *t*-test was used to determine the difference of water quality between the reference and test sites. Benthic macroinvertebrates data were entered into the Ecological Data Application System (EDAS) for the calculation of metrics (Tetra Tech 2000a). Candidate metrics were examined for membership as core metrics to assess biological conditions in large rivers in Thailand. Box and whisker plots were applied to each metric in order to show the difference between the reference and test sites. The metrics with Discrimination Efficiency (DE) higher than 50% in all seasons were selected in this step (Stribling et al 2000). The selected metrics were tested for redundancy using Spearman's correlation coefficient. Based on the metric combinations, the correlation coefficient (*r*) > 0.85 was considered highly redundant. Only one metric from a group redundant metric was selected and included in developing the final index. Moreover, Spearman's correlation was used to compare the water quality class of the DRQ1, CAU index score, WQI^{THAI} (Based on the values of pH, DO, TDS, nitrate, phosphate, turbidity and BOD) and the multimetric system of Thorne & Williams (1997).

Two metric scoring methods were used to develop the multimetric index (i.e., DRQ1 and CAU) (Blocksom 2003). 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 a continuous scoring method. In the first method, each metric was scored by creating a value range from a population of the reference sites and a categorical scoring system of 1, 3 and 5 points was developed for each metric. For the second method, to score metrics, the range of values for each metric was standardized on a 100 point scale and each metric value was assigned a score ranging from 0 (worst) to 100 (best) (Tetra Tech 2000b).

Results and Discussion

Physico-chemical parameters and habitat assessment. Almost all of the physicochemical means between the reference and tested sites were not significantly different ($p > 0.05$), except electroconductivity (EC), total dissolved solids (TDS), turbidity, suspended solids (SS) and nitrate-nitrogen, which were significantly different ($p < 0.05$) on all collection dates. It was found that the depths and dissolved oxygen were slightly higher at the reference sites than the tested sites in all seasons, but they were not significantly different ($p > 0.05$) (Table 1). This result corresponds with the study of Gabriels et al (2010), who stated that the values of oxygen concentration usually decrease with increased environmental disturbances. For the results of electroconductivity, it showed lower values in the reference sites than for the tested sites. This result is similar to Blocksom & Johnson (2009) and Li et al (2010). Moreover, the water quality of the disturbed or tested sites were found to be similar to those of Blocksom & Johnson (2009), Robertson et al (2008), Li et al (2010) and Nguyen et al (2014) who showed that physico-chemical values of tested sites increased in nutrient concentrations by non-point source runoff, agriculture and urbanization leading to elevated conductivity, suspension solid, total dissolved solid and turbidity causing extensive sediment deposition. As presented in Figure 2, box and Whisker plots clearly show that the total habitat score in the reference sites was higher than the test sites in all seasons, which similar to the results of Li et al (2010), who revealed that reference sites tended to have low land use intensity. In addition, this result is consistent with the conclusion of Meng et al (2009) that a high total habitat score was positively related to riparian cover, aquatic vegetation and substrates, which directly involved the diversity of benthic macroinvertebrates.

Site classification. The selection of reference site criteria identified 24 of 77 sites as reference sites in 2012 to 2013. Most reference sites were located in the upper reach of the Phong River, where sites were generally less disturbed including PO01, PO02, PO03 and PO04. Most of the lower reach of the Phong river sites and the most of the Cheon River sites were considered as test sites. According to the reference sites ordination, the illustration of CCA revealed strong separation among the reference and tested sites; moreover, the reference sites tended to aggregate in an ordinal space (Figure 3). This study has shown that reference site ordination has a similar pattern to the previous studies of Blocksom & Johnson (2009); Boonsoong et al (2009); Li et al (2010) and Uttaruk et al (2011). The mean values of BOD_5, TDS, nitrate-nitrogen, EC, chlorophyll a and ammonia-nitrogen were higher in the tested sites, which indicates anthropogenic disturbances. The tested sites are heavily polluted, which is directly related to the surrounding intensive agricultural and industrial areas.

Table 1

Mean±SD of physico-chemical parameters in the reference sites and tested sites in the cool, hot and rainy seasons of 2012-2013

Parameter	*Cool season*		
	Reference (n = 24)	*Test (n = 51)*	*p value*
Depth (m)	2.61±3.46	1.49±1.84	ns
Velocity (m s^{-1})	0.13±0.07	0.14±0.12	ns
Water temperature (^oC)	26.17±1.28	27.34±2.01	0.00*
Dissolved oxygen (mg L^{-1})	5.05±1.13	5.90±1.29	0.00*
BOD_5 (mg L^{-1})	1.10±0.42	1.42±0.40	0.00*
pH	7.03±0.39	7.08±0.45	ns
Electroconductivity (µs cm^{-1})	165.12±19.93	247.73±158.84	0.00*
TDS (ppm)	92.37±18.32	140.00±103.26	0.00*
Turbidity (FAU)	7.92±4.69	29.76±19.48	0.00*
Suspended solids (mg L^{-1})	5.16±2.61	19.13±13.45	0.00*
Nitrate-nitrogen (mg L^{-1})	0.18±0.07	0.40±0.26	0.00*
Ammonia-nitrogen (mg L^{-1})	0.05±0.02	0.07±0.81	ns
Orthophosphate (mg L^{-1})	0.11±0.05	0.20±0.19	0.00*
Chlorophyll a (µg L^{-1})	2.03±1.24	3.07±4.79	ns
	Hot season		
	Reference (n = 24)	*Test (n = 54)*	*p value*
Depth (m)	2.41±2.95	1.15±1.53	ns
Velocity (m s^{-1})	0.14±0.06	0.14±0.07	ns
Water temperature (^oC)	28.88±1.72	29.70±2.17	ns
Dissolved oxygen (mg L^{-1})	4.92±0.55	5.02±0.98	ns
BOD_5 (mg L^{-1})	1.38±0.48	1.51±0.50	ns
pH	7.97±0.37	8.06±0.53	ns
Electroconductivity (µs cm^{-1})	167.71±17.80	250.28±167.09	0.00*
TDS (ppm)	83.67±9.08	124.93±83.11	0.00*
Turbidity (FAU)	8.50±5.99	31.85±19.18	0.00*
Suspended solids (mg L^{-1})	4.92±2.32	17.54±9.88	0.00*
Nitrate-nitrogen (mg L^{-1})	0.22±0.26	0.88±0.94	0.00*
Ammonia-nitrogen (mg L^{-1})	0.08±0.07	0.14±0.30	ns
Orthophosphate (mg L^{-1})	0.14±0.07	0.23±0.51	ns
Chlorophyll a (µg L^{-1})	1.54±0.73	4.96±13.18	ns
	Rainy season		
	Reference (n = 24)	*Test (n = 54)*	*p value*
Depth (m)	3.06±3.75	1.52±1.94	ns
Velocity (m s^{-1})	0.19±0.07	0.16±0.09	ns
Water temperature (^oC)	29.77±0.99	30.56±1.95	0.02*
Dissolved oxygen (mg L^{-1})	5.77±1.41	6.00±1.47	ns
BOD_5 (mg L^{-1})	1.58±0.29	1.60±0.33	ns
pH	7.55±0.32	7.30±0.31	0.00*
Electroconductivity (µs cm^{-1})	161.25±33.25	269.76±88.66	0.00*
TDS (ppm)	94.12±15.64	159.70±49.76	0.00*
Turbidity (FAU)	9.70±6.69	124.04±206.50	0.00*
Suspended solids (mg L^{-1})	6.37±5.32	93.83±149.33	0.00*
Nitrate-nitrogen (mg L^{-1})	0.10±0.07	1.12±1.63	0.00*
Ammonia-nitrogen (mg L^{-1})	0.097±0.096	0.52±1.16	0.00*
Orthophosphate (mg L^{-1})	0.088±0.06	0.198±0.20	0.00*
Chlorophyll a (µg L^{-1})	2.77±0.97	3.94±3.07	ns

*Significant difference at $p < 0.05$; ns - non significant difference.

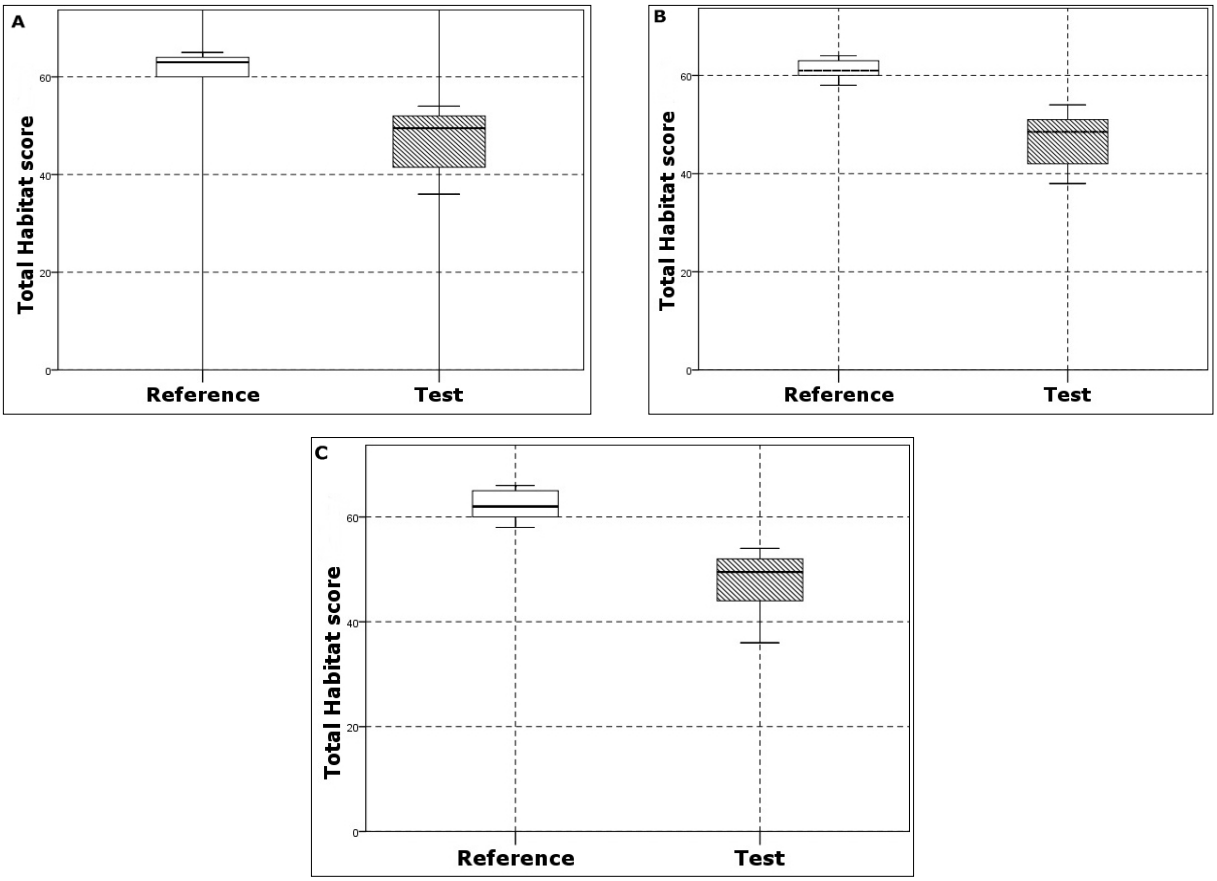

Figure 2. Box and Whisker plots of total habitat scores between the reference and tested sites for (A) cool season; (B) hot season; (C) rainy season.

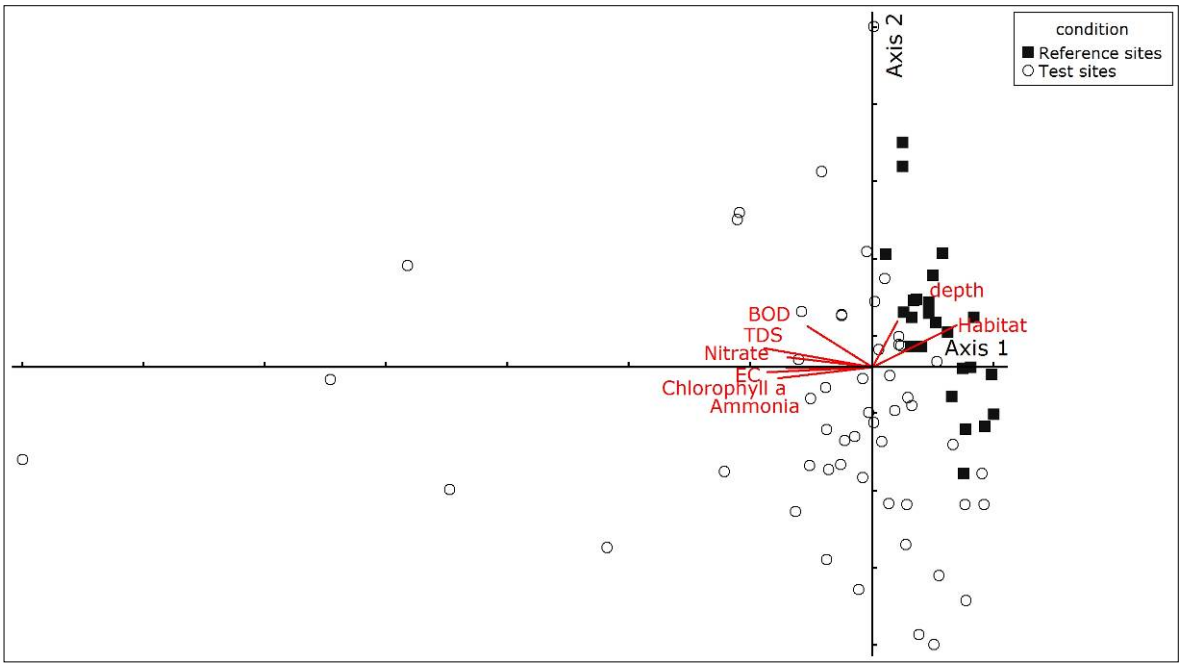

Figure 3. CCA ordination analysis based on physicochemical parameters and benthic macroinvertebrates data between the reference sites and tested sites among the Phong and Cheon Rivers in cool, hot and rainy seasons of 2012-2013.

Selection, calibration of metrics and index development. Of the 45 metrics tested, box plots showed that 19 metrics strongly discriminated as candidate metrics to respond to the anthropogenic stressor. In this study, ten candidate metrics exhibited discrimination efficiencies (DE) of more than 50% in all seasons. Some of the metrics were considered redundant due to a high Spearman's correlation coefficient of greater than 0.85 (Table 2). The percentage of Corbicula (CorbPct) was redundant with percentage of filterer (FiltrPct). In addition, percentage of Trichoptera (TriPct) was redundant with percentage of shredders (ShredPct). Therefore, percentage of Corbicula and percentage of shredders were eliminated as candidate metrics. Eventually, eight core metrics were used to identify for multimetric indices including the number of total taxa, the number of ETO taxa, the number of Coleoptera taxa, percentage of Trichoptera, Margalef's Index, Beck's Biotic Index, percentage of filterers and percentage of burrowers (Figure 4).

All of the eight final metrics could be integrated as the biotic index for the Phong and Cheon Rivers, which may be representative of the different responses to disturbance gradients. This biotic index reflected a balance between different measures, including richness, composition, tolerance and trophic structure of assemblage and more completely reflects overall the ecological quality of rivers (Barbour et al 1995; Karr & Chu 1999). Three richness measures (number of total taxa, ETO taxa and Coleoptera taxa) were chosen as the final index. The total taxa are one of the most commonly used indicators of ecological integrity (Rosenberg & Resh 1993; De Pauw et al 2006). Coleoptera taxa were used successfully in large river bioassessment for developing a regional macroinvertebrate index in the Mid West USA (Blocksom & Johnson 2009). As a result of this study, Coleoptera richness was observed abundantly in the reference sites where it may be related to more available multi-habitats. This finding is consistent with the report of Sharma et al (2013) who stated that aquatic beetles are excellent indicator of habitat quality. The percentage of Trichoptera, a composition measure, was included in the index because of its sensitivity to human disturbance with proportions decreasing in impacted environments (Kashian & Burton 2000; Pereira et al 2012). In addition, a previous study, Boonsoong & Sangpradub (2002) reported that the *Dipseudopsis* caddisfly larvae decreased or disappeared where there was intensive fish cage culture. However, it recovered from organic pollution when the use of fish cage culture was terminated. Also, Margalef's index is retained in composition measure. Margalef's index metric is used in the national river basin monitoring program of Vietnam (Nguyen et al 2014). Beck's biotic index was the only tolerance measure that displayed strong discriminatory power from the box plot. This metric was also used in streams and wetlands in Thailand (Boonsoong et al 2009; Getwongsa et al 2010; Uttaruk et al 2011; Phaphong & Sangpradub 2012). It may be a suitable metric for relation to human disturbance in this ecoregion because this metric showed high weighted sum of intolerant taxa in the reference sites. The percentage of filterers was one of metrics used to construct the multimetric in large river. From this study, filterers including *Physunio* sp. and *Scabies* sp. were obviously higher when sedimentation increased among the tested sites. On the other hand, *Corbicula* sp., *Ensidens* sp. and *Limnoperna* sp. decreased with anthropogenic disturbed. This finding was supported by the study of Klemm et al (2003) and Huang et al (2015), who mentioned that percentage of filterers is best expressed as relative abundance. Also, filterers were sensitive indicators of water quality, and they were less abundant in the disturbed wetland (Kashian & Burton 2000). For habit measure, percentage of burrowers may be more indicative of fine sediments. Both functional feeding groups and habit generally involved the substrate, which they preferentially feed on and inhabit suspended fine organic matter. This metric was also used in the non-wadeable macroinvertebrate assemblage condition index (NMACI) of the USA (Blocksom & Johnson 2009).

Thus, the biotic index has the advantage of integrating different stressors of pollution. In the present study, eight core metrics were chosen as potential metrics for calibration and final index development. In Tables 3-5, the category scoring range and descriptive statistics for all collection dates are shown.

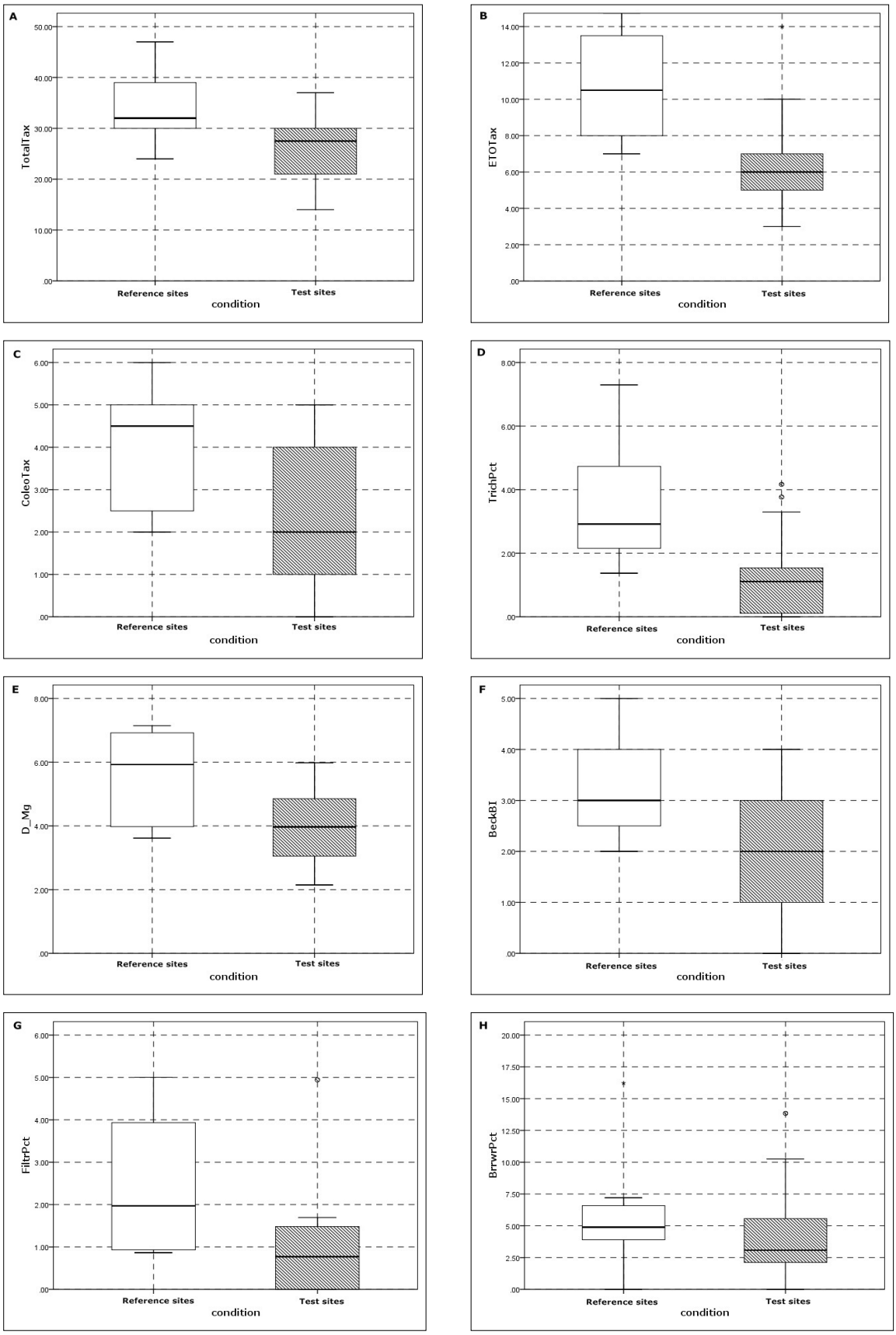

Figure 4. Box and Whisker plots of core metric for index development (A) total taxa; (B) ETO Taxa (Ephemeroptera, Trichoptera and Odonata taxa); (C) Coleoptera taxa, (D) Percentage of Trichoptera; (E) D_Mg (Margalef's index); (F) Beck's Biotic Index; (G) Percentage of filterers; (H) Percentage of burrowers.

Table 2

Spearman's correlation of benthic macroinvertebrates metrics in the reference sites

	TotalTax	*ColeoTax*	*BeckBI*	*CorbPct*[a]	*TrichPct*	*D_Mg*	*BrrwrPct*	*ETOTax*	*FiltrPct*
ColeoTax	0.395								
BeckBI	0.560**	-0.051							
CorbPct	0.394	-0.01	0.124						
TrichPct	0.132	-0.195	-0.138	0.221					
D_Mg	0.713**	0.314	0.337	0.328	0.142				
BrrwrPct	0.129	-0.112	0.082	0.706**	0.204	0.2			
ETOTax	0.524**	0.01	0.638**	0.432*	0.172	0.435*	0.174		
FiltrPct	0.217	-0.135	0.17	0.866**	0.161	0.153	0.701**	0.194	
ShredPct[a]	0.053	-0.237	-0.107	0.189	0.886**	0.138	0.169	0.158	0.177

Marked correlations are significat, [a]Redundancy metrics, *p < 0.05, **p < 0.01;
TotalTax = total taxa, ColeoTax = Coeloptera taxa, BeckBI = Beck's Biotic Index, CorbPct = percentage of Corbicula, TriPct = percentage of Trichoptera, D_Mg = Margalef's index, BrrwrPct = percentage of burrowers, ETOTaxa = Ephemeroptera, Trichoptera and Odonata taxa, FiltrPct = percentage of filterers, ShredPct = percentage of shredders.

Table 3

Descriptive statistics and scores for the core metrics for the cool season

Metrics	*Descriptive statistics*					*Categorical scoring range*		
	Min	*25th*	*Mec*	*75th*	*Max*	*5*	*3*	*1*
Total taxa	24	26	42	46	49	≥26	13-25	<13
ETO Taxa	7	8	10.5	14	15	≥8	4-7	<4
Coleoptera taxa	1	3	4.5	5	6	... a	≥3	<3
% Trichoptera	1.37	2.29	2.92	5.48	7.29	≥2.29	1.14-2.28	<1.14
Margalef's Index	3.62	4.08	5.92	6.94	7.15	≥4.08	2.04-4.07	<2.04
Beck's Biotic Index	2	2.75	3	4	5	≥2.75	1.37-2.74	<1.37
% Filteres	0.87	0.94	1.97	3.96	5.00	≥0.94	0.47-0.93	<0.47
% Burrowers	0	3.77	4.89	6.89	16.22	≥3.77	1.88-3.76	<1.88

a - Considered a weak metric for discrimination and given only two scoring criteria.

Table 4

Descriptive statistics and scores for the core metrics for the hot season

Metrics	*Descriptive statistics*					*Categorical scoring range*		
	Min	*25th*	*Mec*	*75th*	*Max*	*5*	*3*	*1*
Total taxa	29	33	40	48	49	≥33	17-32	<17
ETO Taxa	8	9	12	12.75	14	≥9	4-8	<4
Coleoptera taxa	2	2	4	5.75	6	... a	≥2	2<
% Trichoptera	1.18	3.15	5.28	6.83	9.48	≥3.15	1.57-3.14	<1.57
Margalef's Index	3.37	4.21	6.83	7.4	7.74	≥5.21	2.60-5.20	<2.60
Beck's Biotic Index	3	3	3.5	4.5	6	≥3	1-2	<1
% Filteres	2.24	2.76	3.40	3.78	8.56	≥2.76	1.38-2.75	<1.38
% Burrowers	5.54	5.66	6.67	14.6	15.85	≥5.66	2.83-5.65	<2.83

a - Considered a weak metric for discrimination and given only two scoring criteria.

Table 5

Descriptive statistics and scores for the core metrics for the rainy season

Metrics	*Descriptive statistics*					*Categorical scoring range*		
	Min	*25th*	*Mec*	*75th*	*Max*	*5*	*3*	*1*
Total taxa	24	30	32	41	47	≥30	15-29	<15
ETO Taxa	4	10	11	11.75	12	≥10	5-9	<5
Coleoptera taxa	2	3	4.5	5	6	...a	≥3	<3
% Trichoptera	0.51	2.07	4.9	7.36	9.09	≥2.07	1.03-2.06	<1.03
Margalef's Index	4.36	5.08	5.31	5.68	6.16	≥5.08	2.54-5.07	<2.54
Beck's Biotic Index	0	2.75	3	3.75	4	≥2.75	1.37-2.74	<1.37
% Filteres	0	1.49	1.68	5.51	13.60	≥1.49	0.74-1.48	<0.74
% Burrowers	0.51	4.41	7.87	13.85	20.79	≥4.41	2.20-4.40	<2.20

a - Considered a weak metric for discrimination and given only two scoring criteria.

River health assessment of the Phong and Cheon Rivers. As shown in Table 6, the DRQ1 method and the CAU method were adopted for the development of the metric scoring criteria. In addition, the range of index scores between the reference and tested sites were compared and indicated by box and Whisker plots. The results of the box and Whisker plots, support the effective final index, which clearly discriminates between the reference and tested sites in all seasons as shown in Figure 5. In this study, the narrative assessment was divided into 4 category classes based on the range of index values among all of the reference sites. These ordination rating categories were used to assign impairment ratings to all of the sampling sites. Most of the reference sites were rated "Excellent" or "Good" using both index scores. Furthermore, the results of the assessment show that for the tested site population, each method also showed similar class results as "Good" and "Moderate", "Poor". However, the rating dissimilarity for the sampling sites between the DRQ1 and CAU scoring was 31%. The results show that most sites were in a moderate condition.

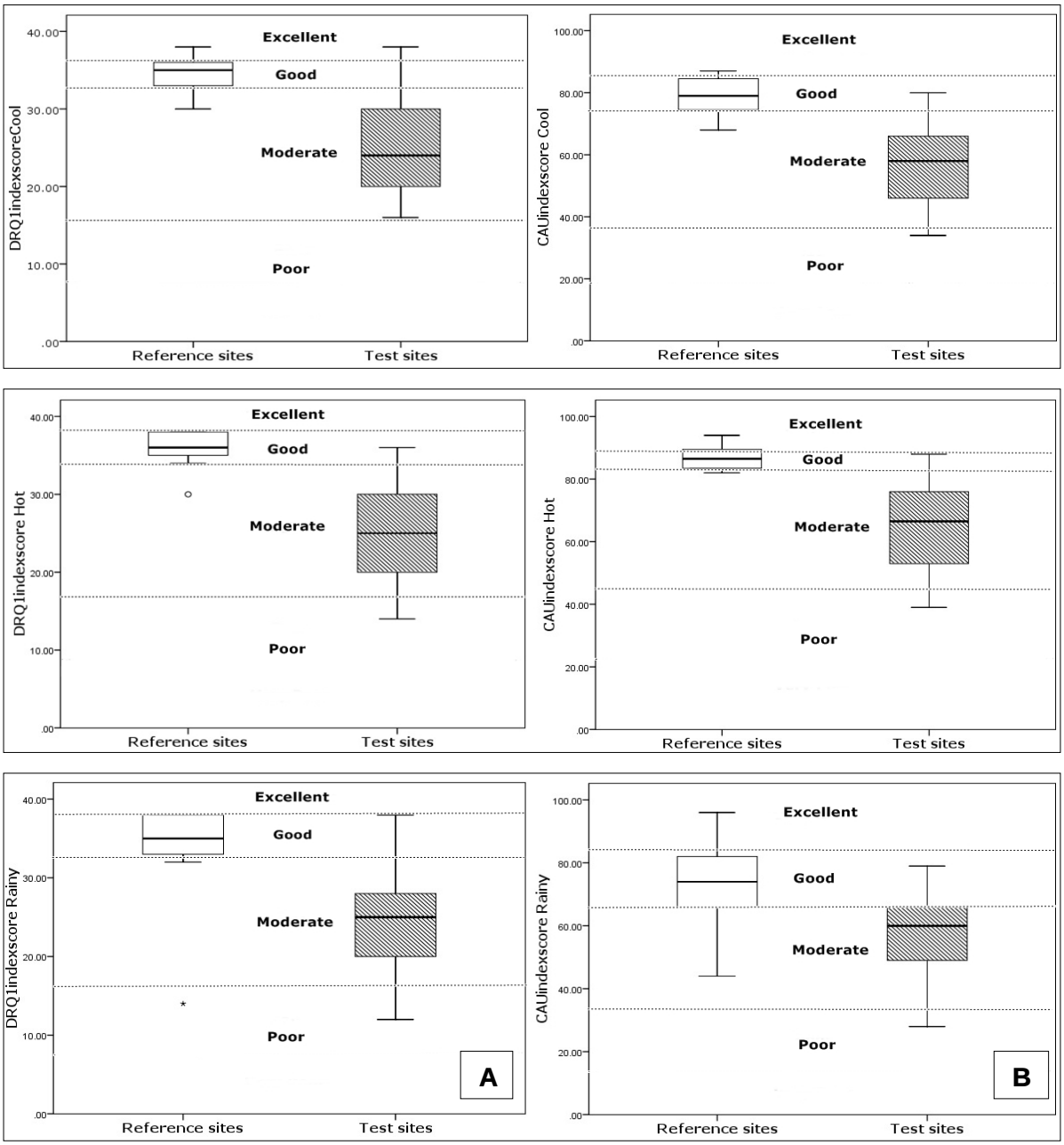

Figure 5. Box and Whisker plots comparing the final index scores between the population of the reference and tested sites (A) DRQ1 index scores; (B) CAU index scores.

Table 6

Definitions of narrative assessment using index values based on core metrics

Narrative assessment	*Percentile of reference index value*	*DRQ1 index score*			*CAU index score*		
		Cool	*Hot*	*Rainy*	*Cool*	*Hot*	*Rainy*
Excellent	≥75th	36	38	38	85-100	89-100	84-100
Good	≥25th	32-35	34-37	32-37	74-84	83-88	65-83
Moderate	<25th	16-31	17-33	16-31	37-73	42-82	33-64
Poor	-	<15	<16	<15	<36	<14	<32

We developed the multimetric index for assessing water quality in the Phong and Cheon Rivers. The index scores of the DRQ1 and the CAU methods worked well in the evaluation of the environmental stressor of the study sites and showed results similar to those of previous studies (Blocksom 2003; Boonsoong et al 2009; Getwongsa et al 2010; Uttaruk et al 2011; Phaphong & Sangpradub 2012). The findings from the DRQ1 method and the CAU method showed similar results. Spearman's correlation analysis indicated that DRQ1 index scores were highly correlated with the CAU index score ($r = 0.751$, $p < 0.05$) (Table 7). Moreover, we also examined the correlation between the CAU index score and WQI^{THAI} (Thai water quality index), which consistently reveals river health ($r = 0.652$, $p < 0.05$). However, the correlations between the DRQ1 method and the WQI^{THAI} were slightly different ($r = 0.548$, $p < 0.05$). From the result of Spearman's correlation analysis, the CAU index score shows a relatively higher correlation with WQI^{THAI} than the DRQ1 index score. This may be the reason why the CAU performed the best for assessing the Phong and Cheon Rivers' healths. However, this result is consistent with the finding of Blocksom (2003), who reported that the CAU method gave the best overall index. According to the results of the early multimetric in Thailand, Ghana and Brazil of Thorne & Williams (1997), the water quality classes of the Phong and Cheon Rivers were categorized having as the same trend with the DRQ1 and CAU methods, with DRQ1 and CAU index scores correlated with the early multimetric, $r = 0.630$ and $r = 0.610$, $p < 0.01$, respectively. However, there was a slight difference between DRQ1, CAU and the early multimetric, with some families of caddisflies including Family Dipseudopsidae and Family Ecnomidae which are important taxa in the Phong and Cheon Rivers, but are absent from the early multimetric. This may be because the early multimetric was developed in streams and wadeable rivers, but it was also comprised of richness, enumerations, diversity and similarity loss, biotic indices and functional feeding, whereas this study focused on non-wadeable rivers in the northeastern, part of Thailand and is composed of richness, composition, tolerance, functional feeding groups and habit measures. Thus, the use of DRQ1 and CAU indices that were developed from the local taxa and were established in non-wadeable rivers, is likely to be a suitable approach for this region.

Table 7

Spearman's correlation of DRQ1, CAU, WQI and multimetric of Thorne & Williams (1997)

Index	*CAU*	*DRQ1*	*WQI^{THAI}*
DRQ1	0.751*		
WQI^{THAI}	0.652*	0.548*	
$Multimetric^{Thorne\ \&\ Williams}$	0.610**	0.630**	0.405**

Marked corelations are significant: *$p < 0.05$, **$p < 0.01$.

For the river quality classes, the study sites were categorized into "Excellent" (8 sites), "Good" (26 sites), "Moderate" (37 sites) and "Poor"(6 sites) based on the CAU index score. Most reference sites were rated "Excellent" and "Good" with most located in the upper reach. However, most of the tested sites in the lower reach were categorized into "Moderate" and "Poor" water quality, except some tested sites which were rated "Good". These sites may be affected by intensive agriculture and urbanization. Additionally, these results were in line with the previous study of Hanjavanit & Tangpirotewong (2007), who noted that the study sites below the Ubolratana reservoir were classified as less impacted

sites, whereas, the study sites were surrounded by intensive urban areas and were the zone below the pulp and paper mill factory and the zone below the sugar mill factory, where were impacted sites. Also, these results were similar to those of the Liao River in the Northeast of China revealing that upstream of the tributaries was least impaired by human disturbance resulting in the water quality at these sites being in “Excellent” and “Good” conditions. Moreover, the impaired sites with “Moderate” and “Poor” water quality were located downstream, which was heavily polluted (Meng et al 2009). Also, these results corresponded to the findings of Nguyen et al (2014) who applied a multimetric index to assess the water quality in the Cau River in Vietnam from very good (upstream) to bad (downstream). Both indices development (the DRQ1 and CAU scoring methods) performed well similar to the overall index to evaluate condition classes (Barbour et al 1999; Tetra Tech 2000b). In addition, Resh & Jackson (1993) indicated that the index score needed to be calibrated in other ecoregions for different impact types. From the current study, the water quality based on the biotic index was clearly related to anthropogenic disturbances. The degraded water quality was observed along the upper reach to the lower reach of the Phong and Cheon Rivers. The index presented here was successful at discriminating reference sites from the tested sites. In addition, it classified the water quality class of the Phong and Cheon Rivers.

Conclusions. The development of a biotic index using the multimetric approach is shown to be practical for rapid bioassessment protocols, which can be used to assess the river health for the Phong and Cheon Rivers in the Northeast of Thailand. Eight core metrics reflected the anthropogenic disturbance gradient. The water quality classes of the Phong and Cheon Rivers were categorized into excellent, good, moderate and poor based on the DRQ1 and CAU scoring methods. The results of water quality in the reference sites showed less impacted conditions. We recommend that more reference sites or best attainable conditions to evaluate the river health should be added and selected. In addition, the index score should be tested for validation and robust implementation in other ecoregions in further studies to support the use of this approach as an effective biomonitoring tool.

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