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Development of rapid bioassessment approaches using benthic macroinvertebrates for Thai streams

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Abstract Thailand currently lacks formal bioassessment approaches and protocols to assist management decisions for water quality. The aim of this research is to develop a practical method of rapid bioassessment for a professional level by using benthic macroinvertebrate assemblages for streams in Thailand. Eleven reference and nine test sites were sampled in the headwater streams of the Loei River and adjacent areas to explore the development of a practical protocol. Specific physico-chemical parameters were selected to provide ecological information supplemental to the biological indicators. The biological research was designed around the USEPA Rapid Bioassessment Protocols (RBPs) using the multihabitat approach. Four fixed-count subsamplings (100, 200, 300 and 500 organisms) were randomly conducted using a standardized gridded pan to evaluate an appropriate level for bioassessment in Thai streams. A 300 organism subsample is adequate for bioassessment purposes in Thai stream (evaluated by calculating dissimilarity values and ordination techniques). A systematic selection of candidate reference sites, metric selection, and index calibration was part of this research. Multimetric and multivariate analyses were examined as a foundation for bioassessment in Thailand. The multimetric approach appears to be more practical for a rapid bioassessment technique. Nine core metrics were identified for biological index score including number of total taxa, Diptera taxa, Ephemeroptera, Plecoptera, Trichoptera, and Coleoptera taxa, (%) Plecoptera, (%) Tolerant organisms, Beck's Biotic Index, (%) Intolerant organisms, Shredders taxa and Clingers taxa were calibrated for the final index. As a result of multimetric and multivariate analyses, family level identification data effectively discriminated reference condition and broad-scale environmental gradients. Hampered by incomplete taxonomic knowledge of benthic macroinvertebrates in Thailand, family-level identification may be sufficient taxonomic resolution for rapid bioassessment in Thailand.

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Introduction

The rapid assessment approach is widely practice in conducting bioassessments, regardless of whether the analysis is rooted in multimetrics (Barbour et al. 1999), or predictive models based on multivariate analyses (Norris 1995). Rapid Bioassessment Protocols (RBPs) are a practical technical approach for conducting cost-effective, yet scientifically valid biological monitoring programmes. The intent of the RBPs is to provide reports that can easily be translated to management and public employing environmentally benign procedures (Barbour et al. 1999). The multimetric and multivariate approaches have been used for data analysis in rapid bioassessment. The multimetric approach is the most frequently used by water resource agencies in the USA, because it provides ecological information that is readily translated to water quality managers (Barbour and Yoder 2000; Barbour et al. 2000). Recently, this approach has been widely used in several other countries (Thorne and Williams 1997; Ofenböck et al. 2004; Vlek et al. 2004; Morse et al. 2007). Similar to the multimetric approaches, multivariate approaches rely on statistical analyses to compare between patterns observed at a test site and pattern expected in the absence of human impact (Norris 1995). RIVPACS (River InVertebrate Prediction And Classication System), AusRivAS (Australian River Assessment System), BEAST (BEnthic Assessment of SedimenT), and ANNA (Assessment by Nearest Neighbour Analysis) are the typical predictive modeling techniques for biological assessment. These multivariate techniques are used widely in Europe and Australia (Reynoldson et al. 1997; Davies 2000; Simpson and Norris 2000; Clarke et al. 2003; Linke and Norris 2005).

The scientific literature supports the contention that freshwater benthic macroinvertebrates are most commonly used for aquatic biological surveys to assess environmental condition in the United States, Europe, Australia and other countries (Resh and Jackson 1993; Resh et al. 1995; Norris 1995; Barbour et al. 1995; Clarke et al. 2003). The results of scientific research confirmed that benthic macroinvertebrates are good indicators of disturbance in Thai rivers and streams (Thorne and Williams 1997; Mustow 2002). In Thailand, the national standard assessment of water quality is based on only bacteria coliform measurements (Pollution Control Department 1997), which are complementary to chemical measurements and can not reflect cumulative stressors. Currently, some volunteer groups and school students in various parts of Thailand use macroinvertebrates as a biological tool to monitor water quality. The procedures are based on a modified method used in Denmark's Blue River, and the result of local research (Kanjanavanit and Moonchinda 2002). However, the official national standard method on using macroinvertebrates as key biological indicators has not been established.

The aim of this research is to develop a practical method of rapid bioassessment by using benthic macroinvertebrate assemblages for streams in Thailand. A preliminary comparison of single habitat and multi-habitat approaches was conducted to seek an appropriate method for Thai streams. But the representative sample of single habitat approach is limited (Barbour et al. 1999). Multi-habitat approach seem to be suitable for sampling a wide variety of stream types (Barbour et al. 2006). Therefore, the multihabitat technique was used throughout this study as being the most appropriate for the diversity of stream in Thailand. In the present study, the methods are designed around the U.S. Environmental Protection Agency (USEPA) RBPs using the multihabitat approach (Barbour et al. 1999). To refine the method for use in Thailand, we conducted a systematic selection of candidate reference sites, tested various technical elements such as level of subsampling, metric selection, and index calibration. Specific physico-chemical parameters were selected to provide ecological information supplemental to the biological indicators (Pollution Control Department 1997). This paper describes the development of a candidate rapid bioassessment approach based on benthic macroinvertebrates for the purpose of determining ecological condition.

Methods

Study area and site selection

The study area was located in Phu Luang Wildlife Sanctuary area (PLWS), Northeastern Thailand (Fig. 1). It covers an area of about 848 km^2. The study area was at latitude 17°00′–17°30′ N, longitude 101°15′–101°45′ E. Twenty sampling sites were chosen in the headwater streams of the Loei River and adjacent catchments. The index periods were chosen to follow seasonal patterns in Thailand (hot, wet, and cold seasons). Water quality and macroinvertebrate samples were collected seasonally for a period of 2 years, which included cold season (November 2004 and November 2005) and hot season (March 2005 and March 2006). High natural seasonal disturbance, especially flooding occurs during the wet season. Then, no samples were collected in wet season. The sites were divided into three catchments, which originated in the PLWS as: (1) the Loei River,

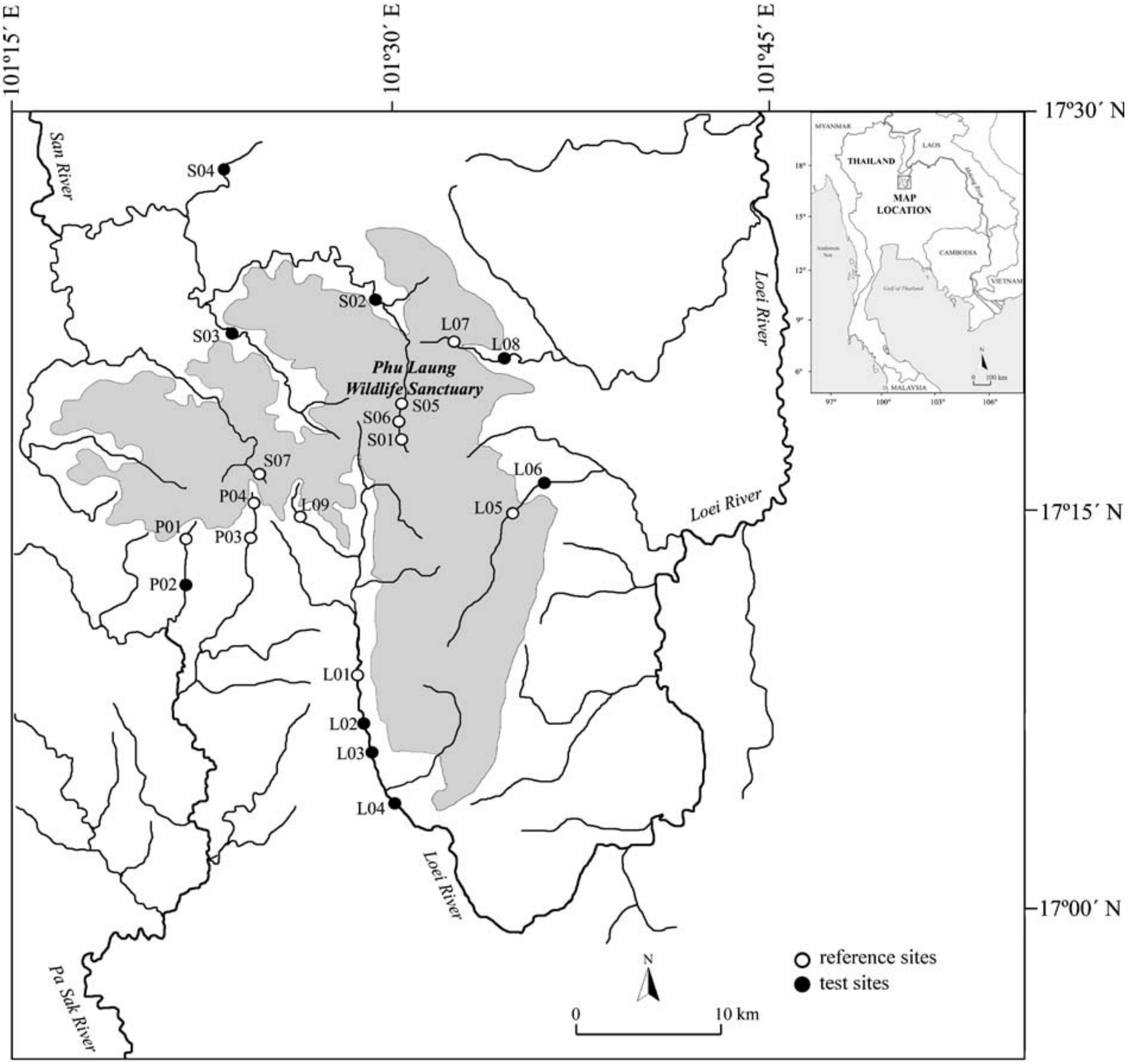


Fig. 1 Map of study area showing the distribution of sampling sites

which joins the Mekong River in Chaing Kan district, Loei province, (2) the San River is a tributary of the Huang River, which joins the Mekong River in Tha Li district, Loei province, and (3) the Pa Sak River, which is the most important watershed in the central part of Thailand. Reference sites represented as unimpaired or least impaired a condition as possible (mostly located in the PLWS); whereas, test sites were known to be influenced by varying levels of anthropogenic stressors. Agriculture is the dominant activity throughout the watershed. Crop production is the major agricultural activity in the Loei River watershed and comprises upland rice, maize, groundnut, beans, and cassava. Typical riparian vegetation consists of *Homonoia riparia* Lour. and *Elaeocarpus hainanensis* Oliv. The streams of all reference sites flow through mixed deciduous forest. The composition of streambed substrates is diverse and characterized by boulder, pebble, gravel, sand, leaf litter and relative high portion of cobble.

Field and laboratory methods

Eleven water quality parameters were measured along with the collection of benthos at each of the sampling sites. In situ measurements included dissolved oxygen (mg l^{-1}) and water temperature (°C) with a YSI Dissolved Oxygen meter Model 57, pH, with the sension™1 Portable pH meter, conductivity (μS cm^{-1}) and total dissolved solids (mg l^{-1}) with Fisher Scientific method 09-326-2, nitrate (mg l^{-1} NO_3^-–N, ascorbic acid method), orthophosphate (mg l^{-1} PO_4^{3-}, cadmium reduction method), suspended solids (mg l^{-1}), and turbidity (FAU) were measured using the Hach DR/2010 spectrophotometer model 49300-00, BOD_5 (mg l^{-1}) was determined as the difference between initial and 5-day oxygen concentrations in bottles after incubation at 20°C and chlorophyll *a* (μg mg l^{-1}) was measured with an extracted-methanol method (APHA, AWWA, WPCF 1998). The physical properties of habitat at each site were assessed using a format recommended by the USEPA (Barbour et al. 1999). The habitat assessment was performed on the sampling reach as the biological sampling. The visual-based habitat assessment technique was evaluated for each parameter (Barbour et al. 1999). The assessment of the habitat includes an evaluation of the variety and quality of the substate, channel morphology, bank structure, and riparian vegetation.

Macroinvertebrates were collected from each site using a D-frame dip net (0.3-m wide, 500-μm mesh). Macroinvertebrate sampling followed the RBPs of the USEPA (Barbour et al. 1999) using the multi-habitat approach. A total of 20 kicks was collected proportionately from all major habitat types over the length of the reach. For example, if the habitat in the sampling reach is 50% of cobble, then 50% or 10 kicks should be taken in cobble substrate (riffle/run) habitats. Contents of all 20 kicks were composited into a single sample and preserved in 70% ethanol. Before subsampling, each sample was rinsed in a 500-μm mesh sieve and large organic material was discarded. Benthic samples were spred across a standardized gridded pan (30 × 36 cm) with 30 grids (6 × 6 cm). Grids were selected for subsampling using a random numbers table. Material (detritus and macroinvertebrates) was removed from grids using forceps. Four fixed-count subsampling (100, 200, 300 and 500 individuals) levels were used for comparison. These organisms from the sorted subsamples were identified to the lowest possible taxon, usually genus or species, except for Collembola, Coleoptera, Diptera, Odonata (family); Acarina (order) and Oligochaeta (class). Identification were based on the reference text “Identification of Freshwater Invertebrates of the Mekong River and Tributaries” (Sangpradub and Boonsoong 2006). Those individuals were assigned to operational taxonomic units (OTUs). The level of the OTUs varied from species to class, but 74% of OTUs were genera and species.

Data analysis

Tolerance values of taxa were assigned based on available sources (Morse et al. 1994; Mustow 2002), and distribution in reference and test sites. Benthic taxa (the lowest taxonomic level) with tolerance values ≤ 3 were considered *intolerant*, whereas those with values ≥ 7 were considered *tolerant*. These assignments were made to test metrics of sensitivity to perturbation.

One-way ANOVA was used to determine if metric values differed significantly as a function of subsample size and differences among the collection date. Independent-sample *t*-test was performed on physico-chemical data to test for differences between reference and test sites. We followed the procedure described by Barbour et al. (1999) for selection of metrics and index development. Metrics were calculated within the Ecological Data Application System (EDAS). EDAS is designed to facilitate data analysis, particularly the calculation of biological metrics and indices (Tetra Tech 2000a). Candidate metrics were examined for membership and applicability as core metrics to assess biological condition in Thailand streams. Overall metric sensitivity was evaluated by comparing the values between reference and test sites. Box and whisker plots were used to determine an appropriate suite of metrics that displayed strong discriminatory power. The decision was based on examination of the 25th percentile, median, and 75th percentile values of the reference site population for each metric. This comparison can be expressed numerically by its Discrimination Efficiency (DE; Stribling et al. 2000). Pearson's correlation analysis was used to select metrics for the index. Metric combinations that results in correlation coefficient >0.85 were considered highly redundant. The discrete (categorical; Barbour et al. 1996) and the continuous scoring method (Tetra Tech 2000b) were used to develop the metric scoring criteria (Table 1). The software PC-ORD version 4 was used for all multivariate analysis. Multivariate analysis of macroinvertebrate assemblage data was conducted by clustering and ordination methods. The macroinvertebrate data were transformed to presence/absence format. The difference of taxonomic resolution (family and genus) on bioassessment was examined using both multimetric and multivariate techniques.

Two approaches were used to develop and test the multimetric index. These approaches are summarized in Table 2 (cold season) and Table 3 (hot season) with the descriptive statistics and the corresponding scoring range for 300-count data. In the first approach, the index was developed by creating a scoring range using values from a population of reference sites, for each metric. A categorical scoring system of 1, 3, or 5 points was developed for each metric. The second approach was to develop a single multimetric index, which was calculated by averaging the individual metric values (nine core metrics) for the site using a continuous scoring system. The "DRQ1 index score" represents summing the values of the core metrics with discrete scoring method; whereas, the "CAU index score" represents as summing the core metrics of continuous scoring method and taking the average. Six index configurations were tested to find the metric combination that resulted in the greatest DEs.

Results

Physico-chemical parameters and habitat characteristics

Water chemistry varied generally following the seasonal cycle. The mean values of most physico-chemical parameters were not significantly different among reference and test sites (Tables 4 and 5). The average stream depth is 11.5–44.8 cm and the average width between to 1.4–7.9 m. Mean conductivity is relatively low (78.6–289.2 μS cm^{-1}) and mean pH values range 6.8–7.8 in all sites. Mean concentration of orthophosphate does not

Table 1 Details of each scoring method for metrics that decrease (increase) with disturbance (Blocksom 2003)

Method (reference)[a]	Type of scoring	Range of metric scores	Upper threshold	Lower threshold
CAU	Continuous	0–100	95th (5th) percentile of all sites	0 (100 or maximum possible)
DRQ1 (Barbour et al. 1996)	Discrete (Categorical)	1, 3, or 5	≥ 25th (75th) percentile of reference	Remaining range bisected for scores of 3 and 1

[a] Method codes set as follows: *C* continuous, *D* discrete, *R* reference sites used to set expectations, *A* all sites used to set expectations, *Q*1 25th percentile of reference sites used for expectations, *U* upper expectation set (all sites only)

Table 2 Descriptive statistic (minimum, maximum, and interquartile values) and score for the core metrics for the cold season

Metrics	Descriptive statistics					Categorical scoring range		
	Minimum	25th	Median	75th	Maximum	5	3	1
No. of total taxa	23	32	37	41	49	≥ 32	31–16	< 16
No. of Diptera taxa	3	5	6	7	10	≥ 5	4–3	< 3
No. of EPTC taxa	12	19	23	28	32	≥ 19	18–10	< 10
(%) Plecoptera	0	3.0	4.4	11.6	23	≥ 3.0	2.9–1.5	< 1.5
(%) Tolerant organisms	1.6	9.4	14.9	22.4	79.4	≤ 22.4	22.3–33.6	> 33.6
Beck's Biotic Index	6	14	17	21	24	≥ 14	13–7	< 7
(%) Intolerant organisms	1.9	9.9	15.6	25.6	39.4	≥ 9.9	9.8–5.0	< 5.0
No. of shredders taxa	0	3	4	6	7	–[a]	≥ 3	< 3
No. of clingers taxa	12	16	20	22	25	≥ 16	15–8	< 8

Data are combined for 2004–2005 (*n* = 13)

[a] Considered a weak metric for discrimination and given only two scoring criteria

exceed 0.14 mg l^{-1} PO_4^{3-}. However, water temperature was significantly higher at test sites than reference sites in both seasons. Nitrate and chlorophyll *a* were significantly higher at test sites than reference sites only in the hot season. The physical habitat structure of all reference sites had a good riparian zone and vegetative protection, whereas riparian buffers and bank vegetation of test sites were scarce (Table 6). Habitat score of riparian vegetative zone width and vegetative protection at both left and right banks corresponded with bank stability score. Some slightly impaired test sites maintained good variety of substrate types and velocity. Box plot analysis showed that total habitat score in reference sites higher than test sites in all collection dates (Fig. 2). Discriminatory power of the total habitat score in the cold season was stronger than in the hot season. Pearson's correlation analysis revealed that 24 metrics were positively correlated ($r > 0.33$, $p < 0.01$) with total habitat score (Table 7).

Subsample characteristics

Number of total taxa increased with increasing the subsample size. However, one-way ANOVA revealed that the number of taxa in 300 and 500 count data were not significantly different, but that 300 and 500 count data were highly significantly different from 100 and 200 count data in the cold season. In addition, a fixed count of 200 and above were significantly different from 100 individuals in the hot season for total taxa (Fig. 3).

Table 3 Descriptive statistic (minimum, maximum, and interquartile values) and score for the core metrics for the hot season

Metrics	Descriptive statistics					Categorical scoring range		
	Minimum	25th	Median	75th	Maximum	5	3	1
No. of total taxa	33	35	38.5	42	48	≥ 35	34–18	< 18
No. of Diptera taxa	4	5	7	8	10	≥ 5	4–3	< 3
No. of EPTC taxa	18	21	25	27	33	≥ 21	20–11	< 11
(%) Plecoptera	0.36	3.08	5.61	8.20	16.88	≥ 3.1	3.0–1.5	< 1.5
(%) Tolerant organisms	9.2	14.6	22.5	33.7	54.5	≤ 33.7	33.8–50.6	> 50.6
Beck's Biotic Index	7	13	21	22	28	≥ 13	12–7	< 7
(%) Intolerant organisms	5.5	16.8	20.2	31.3	40.8	≥ 16.8	16.7–8.4	< 8.4
No. of shredders taxa	1	2	4	6	6	–[a]	≥ 2	< 2
No. of clingers taxa	10	15	17.5	22	24	≥ 15	14–8	< 8

Data are combined for 2004–2005 (*n* = 14)

[a] Considered a weak metric for discrimination and given only two scoring criteria

Table 4 Mean ± SD of physico-chemical parameters in reference and test sites in cold season (November)

Parameter	Unit	November 2004			November 2005		
		Reference ($n = 18$)	Test ($n = 27$)	T-test	Reference ($n = 21$)	Test ($n = 21$)	T-test
Width	m	4.32 ± 1.74	5.48 ± 4.48	0.798	6.03 ± 5.23	7.95 ± 4.91	0.075
Depth	cm	10.83 ± 8.28	18.15 ± 8.96	0.121	44.06 ± 21.81	44.79 ± 8.84	0.353
Velocity	m/s	1.25 ± 0.60	1.66 ± 0.92	0.304	0.49 ± 0.32	0.78 ± 0.28	0.004**
Water temperature	°C	20.50 ± 2.10	22.15 ± 3.23	0.204	22.61 ± 1.14	24.40 ± 1.01	0.000**
Air temperature	°C	21.94 ± 1.54	23.28 ± 1.97	0.540	24.71 ± 1.06	28.00 ± 1.47	0.000**
Conductivity	μs/cm	116.44 ± 53.01	115.43 ± 36.44	0.787	82.13 ± 56.94	78.60 ± 31.28	0.618
TDS	mg/l	77.61 ± 35.35	76.86 ± 24.27	0.790	54.89 ± 37.93	52.10 ± 20.47	0.637
pH	SU	6.97 ± 0.39	6.86 ± 0.42	0.636	7.62 ± 0.17	7.36 ± 0.13	0.000**
DO	mg/l	6.96 ± 1.68	7.36 ± 0.76	0.557	8.08 ± 0.39	7.20 ± 0.58	0.000**
BOD$_5$	mg/l	4.27 ± 2.25	3.75 ± 1.81	0.737	1.44 ± 0.53	0.77 ± 0.61	0.000**
Turbidity	FAU	17.61 ± 3.09	7.19 ± 5.25	0.518	39.10 ± 66.33	84.43 ± 140.70	0.119
Suspended Solid	mg/l	9.28 ± 6.11	3.85 ± 1.96	0.917	24.48 ± 46.00	47.48 ± 92.98	0.143
Nitrate	mg/l	0.27 ± 0.19	0.24 ± 0.24	0.813	0.46 ± 0.52	0.80 ± 0.98	0.159
Orthophosphate	mg/l	0.13 ± 0.13	0.07 ± 0.04	0.180	0.13 ± 0.09	0.08 ± 0.07	0.074
Chlorophyll *a*	μg/l	0.38 ± 0.27	0.37 ± 0.38	0.952	0.41 ± 0.31	0.55 ± 0.33	0.172

$^*p < 0.05$, $^{**}p < 0.01$

A fixed count of 100 and 500 showed significantly different among collection date. Percent loss of taxa increases by about 12% between 300 and 200 organism levels, while 21% loss occurs between 200 and 100 counts. At 100 count the average loss of taxa is nearly 50% and appear to be worse than other fixed-count subsamples. Non-metric multidimensional scaling (NMDS) analysis revealed that 300-count data provided more robust separation between reference and test sites compared to that of 500, 200 and 100 counts, respectively (Fig. 4). Dissimilarity values of Sørensen distance and Jaccard distance analysis revealed that the fixed-count of 300- and 500-count data were not significantly different from each other, but both were significantly lower from 200- and 100-count data (Fig. 5). All things considered, 300 individuals appear to be good subsample size for assess-

Table 5 Mean ± S.D. of physico-chemical parameters in reference and test sites in hot season (March)

Parameter	Unit	March 2005			March 2006		
		Reference ($n = 24$)	Test ($n = 24$)	T-test	Reference ($n = 21$)	Test ($n = 24$)	T-test
Width	m	1.44 ± 1.84	3.63 ± 3.17	0.000**	1.57 ± 2.58	2.54 ± 2.74	0.118
Depth	cm	7.94 ± 5.31	11.46 ± 4.62	0.006**	13.22 ± 9.03	16.04 ± 9.32	0.324
Velocity	m/s	0.11 ± 0.21	0.26 ± 0.27	0.045*	0.24 ± 0.13	0.34 ± 0.23	0.075
Water temperature	°C	22.03 ± 1.96	24.75 ± 3.36	0.001**	20.81 ± 2.19	24.94 ± 2.68	0.000**
Air temperature	°C	25.73 ± 4.05	26.05 ± 4.42	0.792	24.79 ± 3.42	29.25 ± 3.45	0.000**
Conductivity	μs/cm	283.17 ± 180.80	206.32 ± 54.21	0.172	162.36 ± 73.44	166.03 ± 68.35	0.499
TDS	mg/l	188.70 ± 120.60	137.42 ± 36.15	0.174	110.43 ± 46.70	110.59 ± 45.57	0.666
pH	SU	7.64 ± 0.42	7.36 ± 0.31	0.012*	7.67 ± 0.51	7.35 ± 0.10	0.011*
DO	mg/l	5.93 ± 2.05	5.45 ± 1.69	0.381	6.78 ± 1.92	6.68 ± 1.40	0.834
BOD$_5$	mg/l	2.26 ± 1.11	2.59 ± 1.08	0.304	0.99 ± 0.32	1.64 ± 1.31	0.027*
Turbidity	FAU	17.67 ± 19.27	18.13 ± 21.64	0.942	3.76 ± 3.77	10.92 ± 12.93	0.007**
Suspended Solid	mg/l	10.04 ± 11.20	10.00 ± 11.19	0.883	0.95 ± 1.40	6.04 ± 8.43	0.003**
Nitrate	mg/l	0.18 ± 0.13	0.38 ± 0.33	0.011*	0.10 ± 0.06	0.39 ± 0.41	0.002**
Orthophosphate	mg/l	0.14 ± 0.14	0.13 ± 0.12	0.931	0.11 ± 0.08	0.08 ± 0.04	0.237
Chlorophyll *a*	μg/l	1.34 ± 2.69	2.24 ± 3.39	0.181	0.04 ± 0.11	0.56 ± 0.94	0.002**

$^*p < 0.05$, $^{**}p < 0.01$

Table 6 Total score of each habitat parameter in reference sites and test sites for each collection date

Habitat parameters	November 2004		March 2005		November 2005		March 2006	
	Reference ($n = 6$)	Test ($n = 9$)	Reference ($n = 8$)	Test ($n = 8$)	Reference ($n = 7$)	Test ($n = 7$)	Reference ($n = 7$)	Test ($n = 8$)
Bank stability (left bank)	54	53	75	47	64	37	66	44
Bank stability (right bank)	56	49	77	46	66	36	68	40
Channel alteration	116	108	158	102	138	82	138	91
Channel flow status	94	117	107	91	112	96	84	90
Embeddedness	92	115	110	99	111	93	98	100
Epifaunal substrate/ available cover	94	116	118	105	113	91	95	100
Frequency of riffles (or bends)	85	110	85	86	105	93	81	93
Riparian vegetative zone width (left bank)	57	37	76	28	70	24	65	25
Riparian vegetative zone width (right bank)	60	29	80	30	70	21	70	22
Sediment deposition	89	90	121	73	100	66	95	64
Vegetative protection (left bank)	58	53	78	48	67	39	68	42
Vegetative protection (right bank)	60	45	80	47	70	30	70	38
Velocity/depth regime	84	121	100	95	103	96	96	95

ment and more cost-effective than the 500-count subsample size. In this study, further analysis of macroinvertebrate data were conducted on 300 fixed-count subsamples.

Selection of metrics

Metrics were selected to represent diverse aspects of structure, composition and richness. Of the 56 metrics tested, 16 metrics were chosen as candidate metrics, which demonstrated discrimination efficiency (DEs) of more than 50%. Surprisingly, the Ephemeroptera, Plecoptera, and Trichoptera taxa richness showed relatively moderate DE, whereas the Beck's Biotic Index performed the best overall. Correlation analysis was run on these 16 metrics to exclude redundant metrics from the index. Those metrics with a correlation coefficient >0.85 were considered redundant and were not used together in any index formulation. Margalef index was redundant with insecta taxa and total taxa, while the metric of Trichoptera taxa was redundant with Ephemeroptera, Plecoptera Trichoptera taxa, and Coleoptera (Table 8). The Plecoptera taxa metric was redundant with intolerant taxa. In addition, the intolerant taxa metric suggested redundancy with Beck's Biotic Index. Therefore, the Margalef index, insecta taxa, Trichoptera taxa, Plecoptera taxa and intolerant taxa

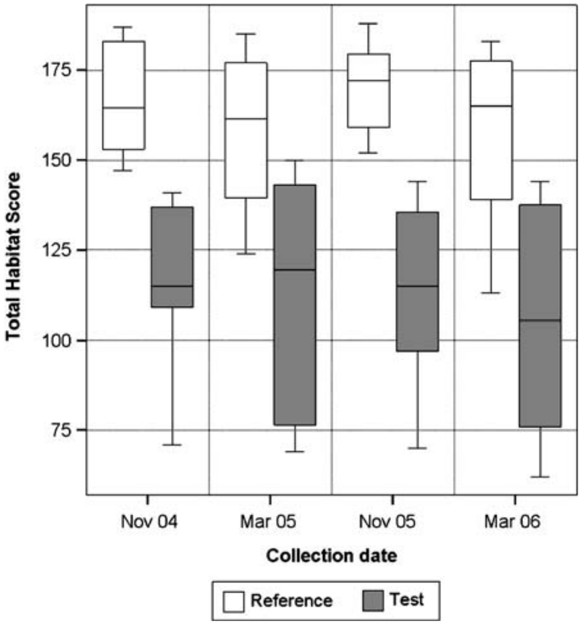


Fig. 2 Box plot of total habitat score of reference and test sites for each collection date

Table 7 Pearson correlation between the benthic metrics and total habitat score in both reference and test sites ($n = 60$)

Category	Metric	r
Richness measures	Total taxa	0.57**
	Ephemeroptera + Plecoptera + Trichoptera taxa	0.62**
	Ephemeroptera taxa	0.34**
	Plecoptera taxa	0.66**
	Trichoptera taxa	0.57**
	Coleoptera taxa	0.45**
	Diptera taxa	0.41**
	Plecoptera + Odonata + Ephemeroptera + Trichoptera taxa	0.60**
	Ephemeroptera + Plecoptera + Trichoptera + Coleoptera taxa	0.65**
	Insecta taxa	0.66**
	Crustacea + Mollusca taxa	0.42**
Composition measures	(%) Ephemeroptera + Plecoptera + Trichoptera	0.35**
	(%) Plecoptera	0.55**
	(%) Chironomidae	−0.29∗
	(%) Domimant taxon	−0.35 ∗ ∗
	(%) Non-insect	−0.42 ∗ ∗
	(%) Crustacea + Mollusca	−0.37 ∗ ∗
Tolerance/Intolerance measures	Intolerant taxa	0.73**
	(%) Intolerant Individuals	0.47**
	Tolerant taxa	−0.51 ∗ ∗
	(%) Tolerant individuals	−0.63 ∗ ∗
	Beck’s Biotic Index	0.73**
Feeding measures	Filterer taxa	0.37**
	Predator taxa	0.32**
	Shredder taxa	0.62**
	(%) Shredder	0.55**
	(%) Collector	−0.33 ∗ ∗
Habit measures	Clinger taxa	0.64**
	(%) Clinger	0.46**
	Climber taxa	0.32*
	Swimmer taxa	0.27*

Marked correlations are significant

*$p < 0.05$, **$p < 0.01$

were eliminated as candidate metrics (Table 8). From this analysis, 9 core metrics were identified as potential metrics for aggregation into an index at genus-level taxonomy.

Calibration of metrics and index development

The results showed that the model calculation of 9 core metrics (number of total taxa, EPTC taxa, Diptera taxa, (%) Plecoptera, (%) Tolerant organisms, Beck’s Biotic Index, (%) Intolerant organisms, Shredders taxa and Clingers taxa) had the strongest DEs for both the DRQ1 and CAU indexes in both seasons. The graphical display of discriminatory power of the indexes for each of the two index periods further supports the ability of the indices to discriminate between the populations of reference and test sites (Fig. 6).

Pearson’s correlation analysis indicated that both indexes were positively correlated with total habitat score (Fig. 7). Both index scores were negatively correlated with increasing nitrate ($r > -0.20$, $p < 0.05$), water temperature ($r > -0.36$, $p < 0.01$) and chlorophyll a ($r > -0.34$, $p < 0.01$). Conversely, there was a positive correlation between dissolved oxygen and index scores ($r > 0.51$, $p < 0.05$). Coefficient of variation [CV, recorded as a (%)] of (%) Plecoptera and (%) Tolerant organisms were the most variable than the other metrics. Variability analysis revealed that macroinvertebrate composition and tolerance/intolerance metrics were more variable both

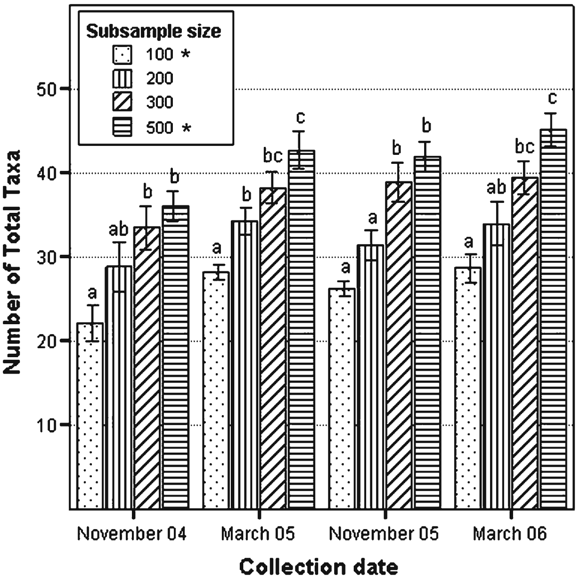


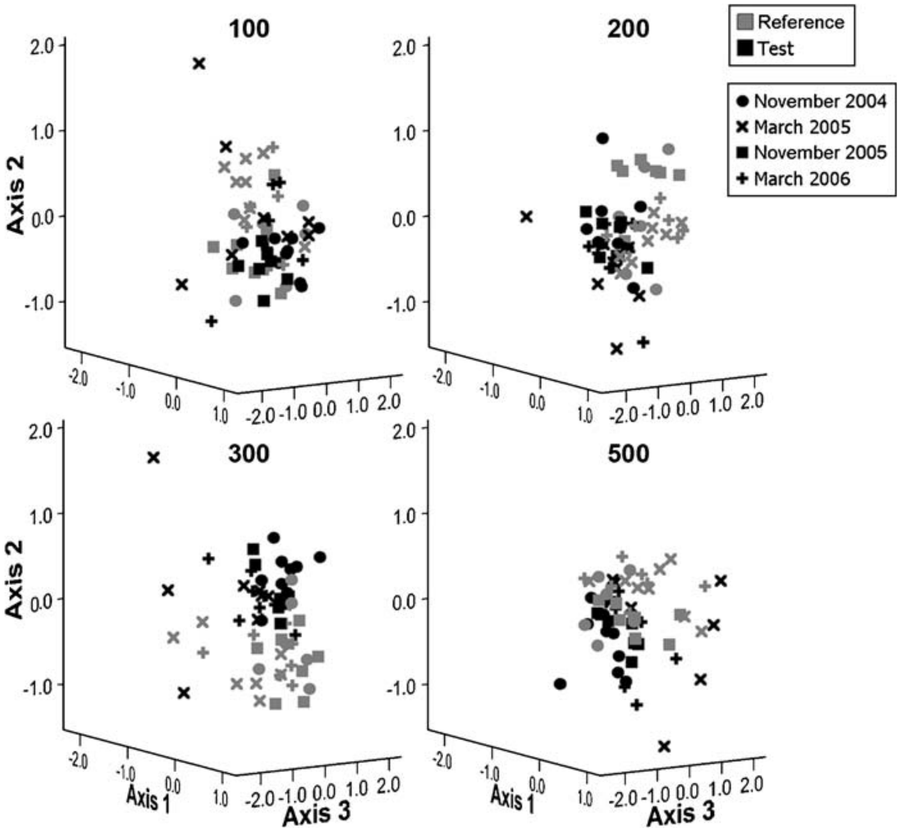
Fig. 3 Mean number of total taxa identified for each subsample across reference sites. *Error bars* are ±1SE. Histograms with the same letter are not significantly different among subsample size. Marked differences are significant among collection date

temporally and spatially than richness metrics and functional feeding group metrics.

Stream bioassessment

The assessment narrative of condition is divided into five categories based on the range of index values among all reference sites. Greater than or equal to the 75th percentile of that range is rated as "Very Good" and greater than or equal to the 25th percentile of that range is rated as "Good". Biological index values from a site compared to the reference condition falling below the 25th percentile are rated in three condition categories as "Fair", "Poor", and "Very Poor", respectively (Fig. 6). These ordinal rating categories were used to assign impairment rating to all sampling sites. The DRQ1 method had narrative assessment proportion results similar to the CAU method at the population of reference site. Approximately 76%

Fig. 4 Three-dimensional NMDS ordinations plots of macroinvertebrate assemblages data among 100, 200, 300 and 500 subsample levels in each index period data



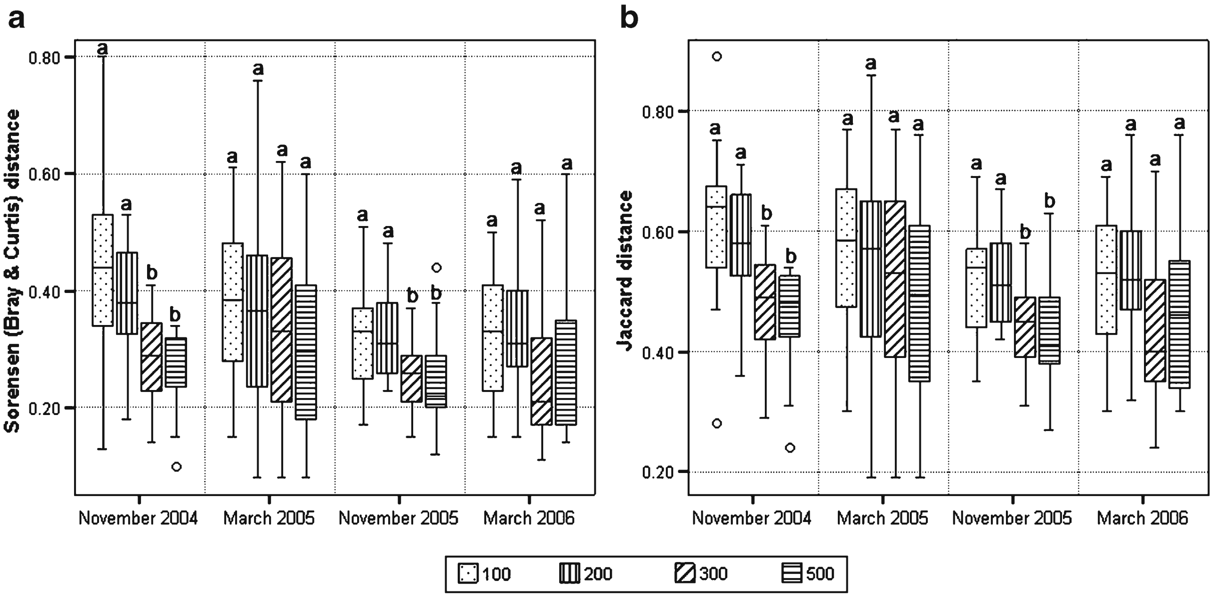


Fig. 5 Box plot of the dissimilarities value (**a** Bray–Curtis distance, and **b** Jaccard distance) between-reference sites among subsample level in each collection date. Subsample size with the same letter are not significantly different

of the a priori reference sites were "Good" and "Very Good" conditions. Moreover, rating similarity of sampling sites among discrete and continuous scoring was 72%. Most of the test sites were categorized as "Fair" (69%) in CAU index score, which were more than with the DRQ1 index score (47%; Fig. 8).

Multivariate analysis

Cluster analysis based on presence/absence data of all samples produced five groups of reaches at the farthest dissimilarity distance. Most of reference sites were separated at an 85% dissimilarity threshold (arrow, Fig. 9). Ordinations of samples resulting from NMDS also showed quite clear separation on two dimensions for most samples between reference and test sites (Fig. 4). Narrative assessment of each sampling site was compared with cluster analysis results. It showed that most of reference sites were rated as "Good–Very Good" in both index scores. Most of the site members of slightly-moderately impaired test sites were rated as "Fair–Good", which were in this rating because they maintained good habitat characteristics. While, members of severely impaired test sites were mostly perceived as "Poor", they were typically located in residential areas or associated with drought conditions. This study revealed that multivariate and multimetric approaches yielded the same results for biological assessment.

Effect of taxonomic resolution on bioassessment

For family level, seven core metrics [number of total taxa, EPTC taxa, (%) Plecoptera, (%) Tolerant organisms, Beck's Biotic Index, (%) Intolerant organisms, and Clingers taxa] were found to be the most robust for metric calibration. This analysis revealed that family-level identification provided DEs as well as genus-level for index calibration. NMDS analysis revealed that both family and genus-levels provided similar pattern to distinguish between reference and test sites (Fig. 10). Moreover, family plus EPT (genus-level) and family-level identification related to stressor values nearly as well as genus-level identification. In the population of reference sites, dissimilarities in values increased with finer taxonomic resolution.

Table 8 Pearson correlation matrix of benthic metrics in reference sites ($n = 27$) at genus-level taxonomy

Variable	Total taxa	Plecoptera taxa[a]	Trichoptera taxa[a]	Diptera taxa	EPTC taxa	Insecta taxa	(%) Plecoptera
Total taxa							
Plecoptera taxa	0.31						
Trichoptera taxa	0.59**	0.34					
Diptera taxa	0.53**	0.25	0.02				
EPTC taxa	0.81**	0.51**	0.87**	0.14			
Insecta taxa[a]	0.99**	0.39**	0.64**	0.53**	0.84**		
(%) Plecoptera	−0.02	0.59**	−0.09	0.02	0.07	0.01	
Margalef Index[a]	0.97**	0.27	0.48*	0.55**	0.69**	0.95**	−0.02
Intolerant taxa	0.48*	0.88**	0.57*	0.33	0.68**	0.57**	0.45*
(%) Tolerant	−0.33	−0.16	−0.28	0.02	−0.38	−0.40*	−0.36
Beck's Biotic Index	0.46*	0.83**	0.65**	0.31	0.67**	0.55**	0.43*
(%) Intolerant	0.10	0.46*	−0.06	0.19	0.03	0.13	0.72**
Predator taxa	0.63**	−0.07	0.00	0.41*	0.24	0.58**	−0.27
Shredders taxa	0.32	0.64**	0.36	0.43*	0.37	0.35	0.47*
Clinger taxa	0.57**	0.64**	0.75**	0.06	0.81**	0.63**	0.18

Variable	Margalef Index	Intolerant taxa[a]	[%] Tolerant	Beck's Biotic Index	[%] Intolerant	Predator taxa	Shredders taxa
Intolerant taxa	0.42*						
(%) Tolerant	−0.36	−0.29					
Beck's Biotic Index	0.40*	0.94**	−0.33				
(%) Intolerant	0.17	0.42*	−0.51**	0.45*			
Predator taxa	0.67**	−0.07	0.06	−0.17	−0.15		
Shredders taxa	0.28	0.70**	−0.10	0.75**	0.39*	−0.26	
Clinger taxa	0.48*	0.66**	−0.42*	0.75**	0.09	0.00	0.35

Marked correlations are significant

[a] Redundancy metrics

*$p < 0.05$, **$p < 0.01$

Discussion

Physico-chemical parameters and habitat characteristics

Instantaneous chemical data collected in this study do not reflect the long-term exposure to biological communities and only represent the water quality at single points in time. However, the physical measurements reflect the patterns of temporal discharge in the streams “”. The results showed that biological index scores were negatively correlated with increasing concentration of nitrate, chlorophyll *a* and water temperature, that is, biological condition decreased. In this study, most of impaired sites were influenced by agriculture land use or forest harvesting, which initial increase in light, nutrients, water temperatures, periphyton biomass and production in streams (Lenat and Crowford 1994; Allan 2004).

Habitat assessment for bioassessment purposes is traditionally done at the sites where the biological sampling occurs. The relationship between habitat assessment and bioassessment based on macroinvertebrates has been demonstrated by others (Barbour et al. 1995; Rabeni 2000). Total habitat score positively correlated with 24 metrics that decrease with impairment. In addition, total habitat scores were strongly correlated with biological index score. This evidence corresponded to many investigators (Lammert and Allan 1999; Karr and Chu 1999; Nerbonne and Vondracek 2001). The pattern of macroinvertebrate distribution in this study could be related to the ability of macroinvertebrate taxa to tolerate environmental conditions associated with agricultural land use.

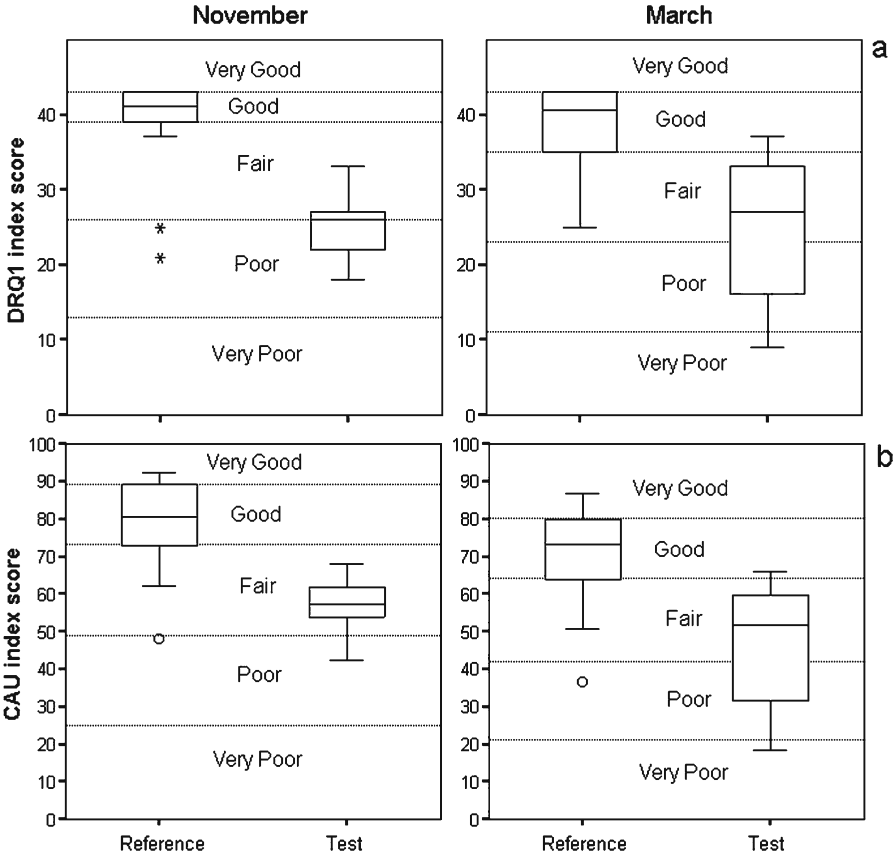


Fig. 6 Box plot comparing the index scores (**a** DRQ1, and **b** CAU) between the population of reference and test sites for the two index periods at genus-level taxonomy and five narrative assessment categories. [a]Method codes set as follows: *C* continuous, *D* discrete, *R* reference sites used to set expectations, *A* all sites used to set expectations, *Q*1 25th percentile of reference sites used for expectations, *U* upper expectation set (all sites only)

Tree cover in the riparian zone appeared to increase EPT, total richness and diversity (Rios and Bailey 2006). As a result of agricultural impact, decreased allochthonous leaf input, greater periphyton growth, and increased sediment input and water temperature are primarily responsible for the change in high gradient streams (Kedzierski and Smock 2001).

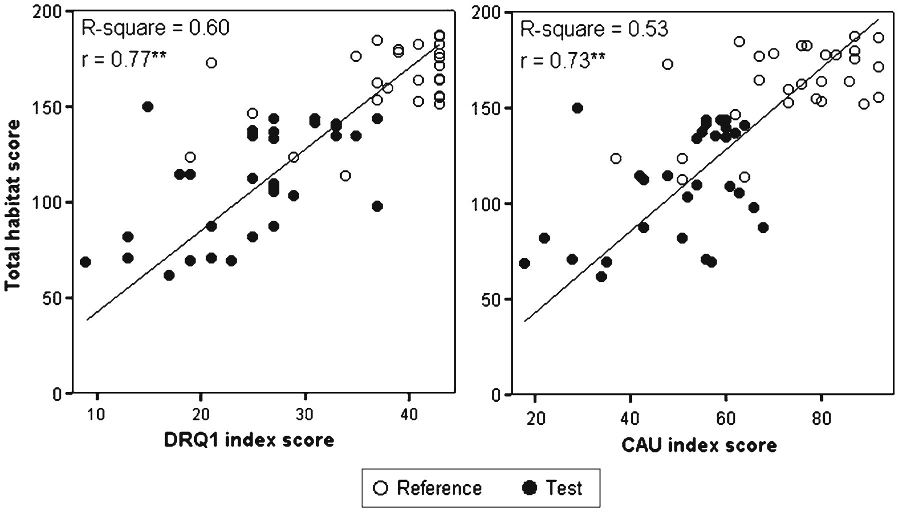


Fig. 7 Scatter plot between DRQ1 index score, CAU index score and total habitat score in reference and test sites

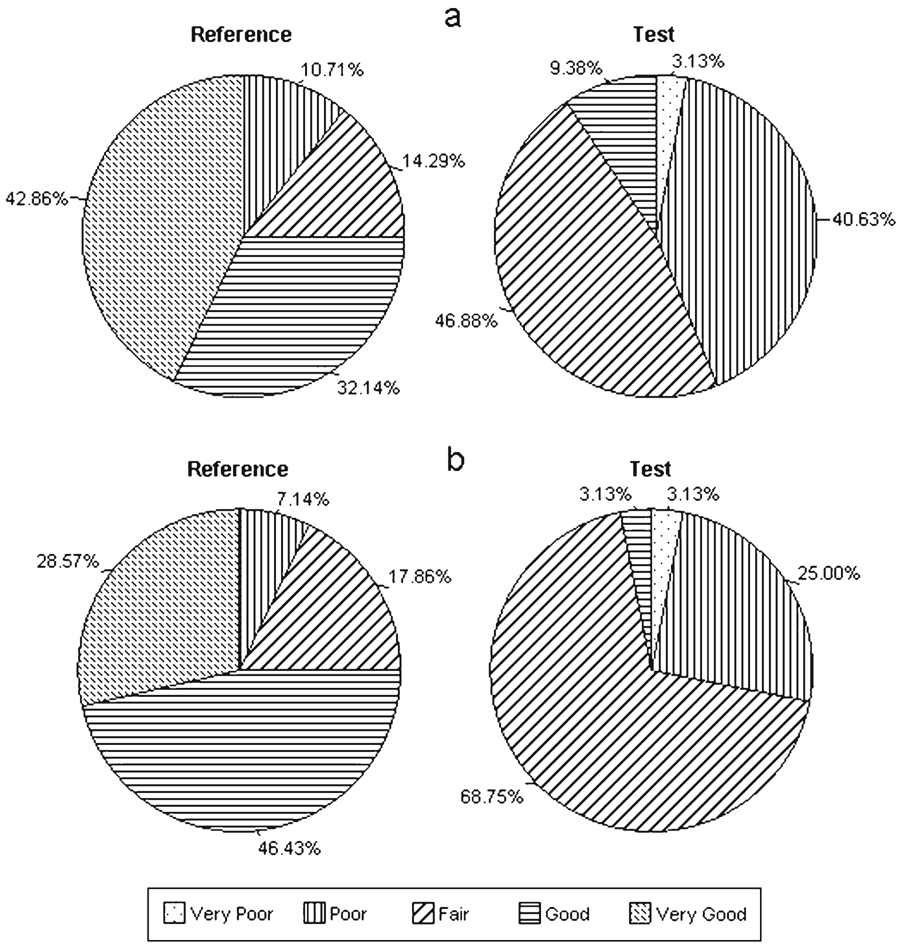


Fig. 8 Pie diageam showing the percentage of narrative assessment of reference and test sites between **a** DRQ1, and **b** CAU methods

Subsample characteristics

This study showed that number of total and EPT taxa increased with increasing the subsample size. These results are consistent with previous observations (Growns et al. 1997; Somers et al. 1998; Sovel and Vondracek 1999) and support the expectation of more taxa as more organisms are included in the sample. Subsampling recommendations in the literature have ranged from 100–300 (Vinson and Hawkins 1996; Barbour and Gerritsen 1996; Larsen and Herlihy 1998; Somers et al. 1998; Barbour et al. 1999), while more recently, the effect of low-count subsampling on bioassessment methods have been examined (Sovel and Vondracek 1999; Doberstein et al. 2000; King and Richardson 2002; Ostermiller and Hawkins 2004). Results from this study showed that the 100-count data performed significantly worse than other fixed-count subsamples. This study supports earlier work that the 100-organism fixed count appeared to be biased for measuring taxa richness in a rapid bioassessment method (Courtemanch 1996; Vinson and Hawkins 1996; Growns et al. 1997; Sovel and Vondracek 1999; Karr and Chu 1999). The results of the discriminatory power calculations revealed that a fixed count of either 300 or 500 organisms provided the strongest discriminatory power of core metric value distributions between reference and test sites. A fixed count of 300 individuals produced higher total unitless scores (i.e., translation of metric value to score) than other subsample sizes. Several earlier papers sug-

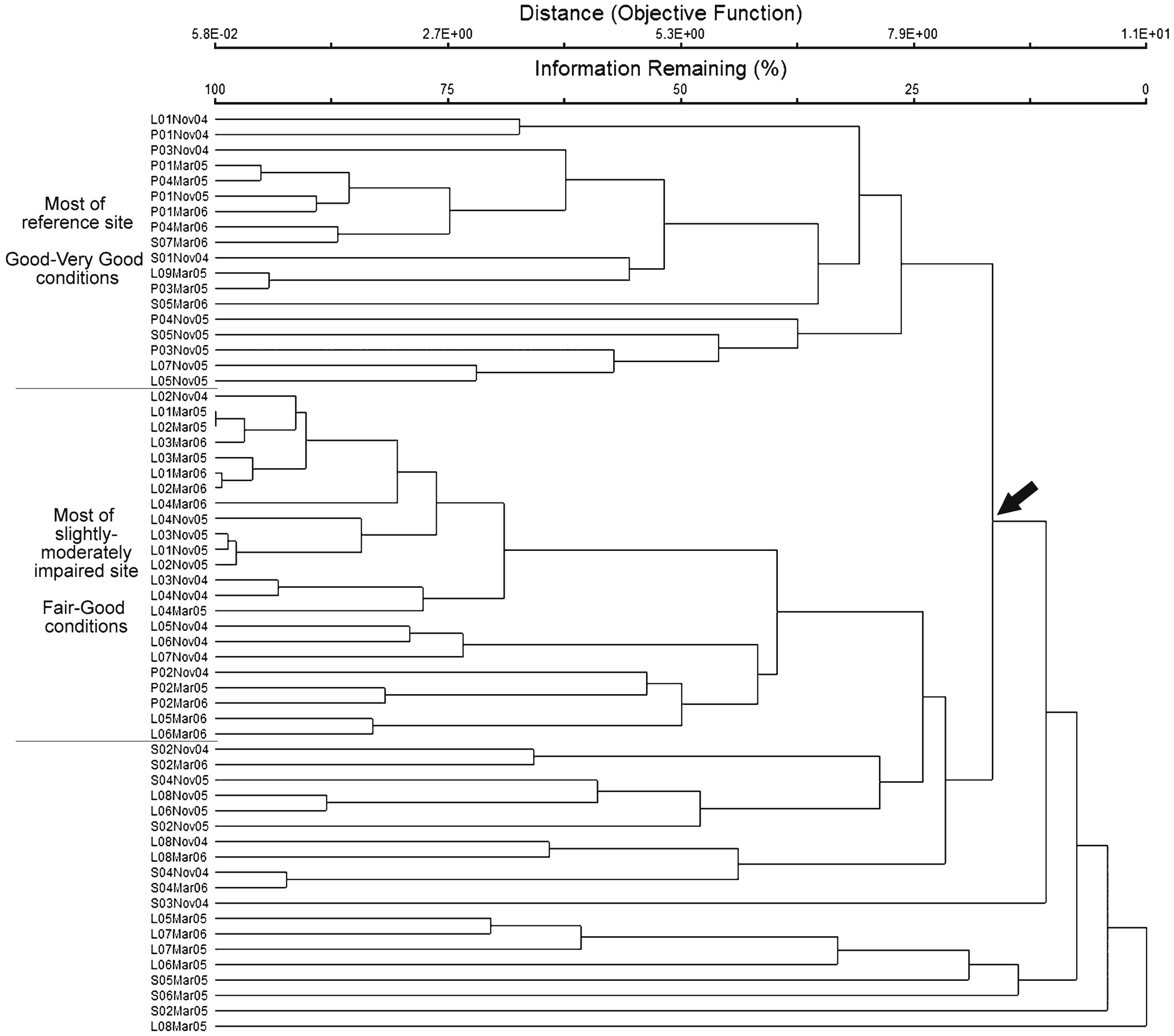


Fig. 9 Dendrogram of cluster analysis of Jaccard distance measure of macroinvertebrate assemblages from 60 samples of the reference and test sites in the Loei River and adjacent catchments, four collection dates

gested that subsamples with >300 individuals are much less sensitive to differences in sample counts than samples with <300 individuals (Vinson and Hawkins 1996; Somers et al. 1998; Sovel and Vondracek 1999). Our study indicated that a 300-count subsampling level provided the best balance between power of resolution and cost effectiveness. Although, Power–Cost Efficiency (PCE) analysis was not performed in this study (as per Barbour and Gerritsen 1996), these results indicated that increasing the fixed count to 500 was not significantly different from a 300-count subsample in detecting impairment. Moreover, an increase in effort and processing costs was associated with the 500-count subsample. Hence, a fixed count of at least 300 individuals is a reasonable indicator of taxa richness for comparative purposes in Thai streams. Therefore, the 300-organism subsample was considered to be sufficient for bioassessment purposes at a professional level for use in Thailand. However, subsample size should be maintained within 20% of the target (Barbour and Gerritsen 1996; Barbour et al. 1999).

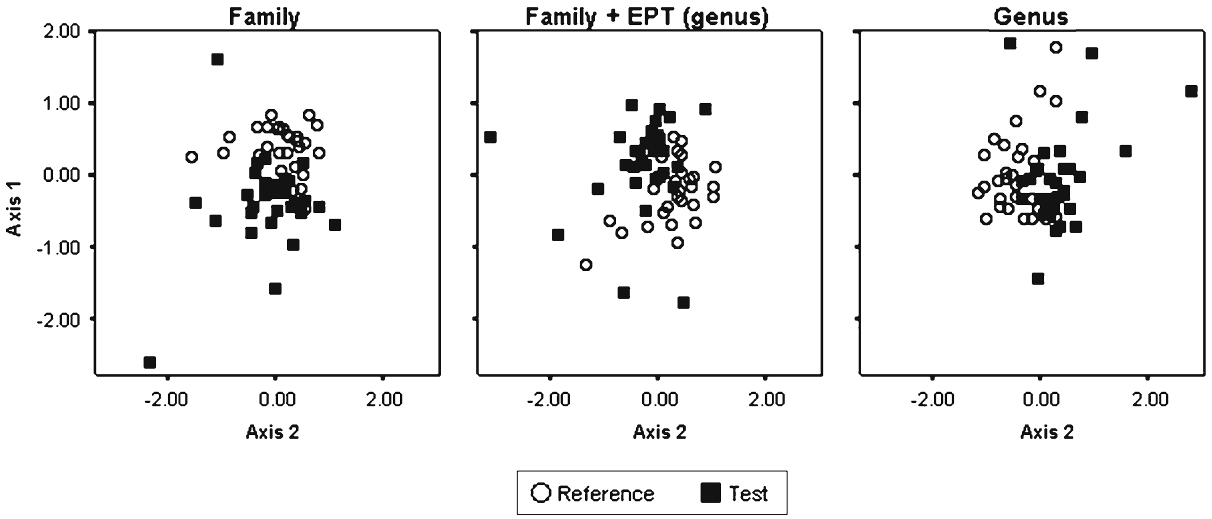


Fig. 10 NMDS ordinations plots of the reference and test sites among taxonomic levels

Calibration of metrics and index development

Metrics were selected by examining graphical distributions (box-and-whisker plots). The 9 core metrics [number of total taxa, EPTC taxa, Diptera taxa, (%) Plecoptera, (%) Tolerant organisms, Beck's Biotic Index, (%) Intolerant organisms, Predators taxa, and Clingers taxa] were aggregated to a final index score. Those metrics represent richness, composition, tolerance and trophic structure of the benthic macroinvertebrate assemblage. Core metrics should be selected to represent diverse aspects of structure, composition and individual health (Barbour et al. 1995). Most multimetric biological indices for aquatic systems comprise eight to 12 metrics (Karr and Chu 1999).

Three richness measures (number of total taxa, EPTC taxa, and Diptera taxa) were retained as components of the final index. Our results indicated that EPT taxa richness was of moderate strength for discriminating impairment. EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa richness has been widely used as indicators of environmental disturbances (Wallace and Webster 1996; Klemm et al. 2002) and is combined within several multimetric indices (Barbour et al. 1999; Ofenböck et al. 2004). Our study showed that a slight increase in EPT richness occurred at slightly impaired test sites thus counfounding assessments. Better results were obtained when Coleoptera was added to the EPT orders; EPTC taxa, therefore was more robust than EPT, alone. EPT was indicated as to be not as good as EPTC in assessments yet only Table 8 shows any data in this regard and both are significant at $p < 0.01$ level. This study found that composition measures were highly variable. Composition measures are known to have high variability (Doberstein et al. 2000; Maloney and Feminella 2006). Three different tolerance/intolerance measures [Beck's Biotic Index, (%) Tolerant organisms and (%) Intolerant organisms] were found to be core metrics. A trisection of the index scoring range (discrete scoring method) and continuous scoring method, which has been well documented (Barbour et al. 1999; Tetra Tech 2000b), was used to distinguish condition classes. The DRQ1 method, with discrete scaling, performed nearly as well overall as the CAU methods. This study corresponds to the conclusion of Blocksom (2003), which was that a continuous scoring method (CAU) performed the best overall for the Macroinvertebrate Biotic Integrity Index (MBII) and tended to result in less index variability. However, each scoring method has some limitations for implementation. Although both the DRQ1 and CAU scoring methods produced the best overall index for this set of metrics and data, further adjustment of the index score is needed for bioassessment in Thailand.

Effect of taxonomic resolution on bioassessment

This study demonstrated that family level identification data were as effective as genus data in characterizing the reference condition and broad-scale environmental gradients. This observation agrees with many studies that showed that variation in assemblage composition along strong environmental gradients can be detected with coarse taxonomy (Choy and Marshall 2000; Metzeling et al. 2006). Thorne and Williams (1997) demonstrated that biological monitoring programs using family-level identification in multimetric indexes appear to have great potential for use in developing countries. Family-level identification may be sufficient taxonomic resolution for rapid bioassessment in Thailand. Furthermore, the knowledge for family-identification and genus-identification of the EPT orders are established in Thailand (Sangpradub and Boonsoong 2006). However, fine taxonomic resolution (the lowest possible taxon identification) may be preferable, particularly for discriminating among multiple stressors, and should be a goal for refinement of bioassessment in Thailand.

Conclusion and recommendation for bioassessment of Thailand streams

In conclusion, this study showed that a multimetric approach with a fixed count of 300 organisms appears to be a good technique for rapid bioassessment protocols in Thai streams. The nine core metrics were calculated to a final index score. Several of the approaches evaluated in this study have been determined to be acceptable as a common framework for freshwater biomonitoring in Thailand. However, recommendations are made to test these protocols in further studies; and further testing and validation of our index should be done in other regions of Thailand to provide support for the use of this approach as a foundation throughout the country. To address watershed management and water pollution, the Thai regulatory system for water resources management and pollution control should focus on biomonitoring tools for water quality assessment. A standard biological assessment program is an important issue to implement in determining the health of streams in Thailand.

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