Vol. 10: 119–130, 2010
doi: 10.3354/ab00274

AQUATIC BIOLOGY
Aquat Biol

Published online August 12

Methods for sampling benthic macroinvertebrates in tropical lentic systems

Lynette H. L. Loke[1], Esther Clews[1], E-wen Low[2], Christina C. Belle[1], Peter A. Todd[1,*], Hans S. Eikaas[3], Peter K. L. Ng[1]

[1]Marine Biology Laboratory, Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Blk S1 #02-05, 117543 Singapore

[2]Technology & Water Quality Office, and [3]Catchment and Waterways Department, Public Utilities Board, 40 Scotts Road, #22-01, 228231 Singapore

ABSTRACT: Reliable quantitative methods for sampling invertebrate communities are critical for effective freshwater biomonitoring. We tested a range of devices and protocols for sampling benthic macroinvertebrates in shallow tropical lakes; this is the first time this has been attempted in Southeast Asia. First, a pilot study to identify a suitable artificial substrate and colonisation period was conducted. Coconut brushes combined with split palm fronds attracted the greatest macroinvertebrate abundance and richness. A colonisation period of 4 wk was sufficient to capture the key macroinvertebrate families and orders. Second, the sampling efficiencies of 7 artificial substrate sampler designs and 2 hydraulic suction devices were compared in rocky and soft-sediment littoral habitats of a reservoir in Singapore. Among the 9 different sampling techniques tested, the samplers containing coconut brushes and split palm fronds again were the most effective at capturing the greatest total abundance and family richness of benthic macroinvertebrates. Variation in community structure among sampler types was largely explained by the abundance of Chironomidae and Polymitarcyidae (Ephemeroptera). Results were similar between sites dominated by 'rocky' and 'vegetated' littoral habitats. This project identified a sampling device suitable for biomonitoring Singapore's lentic environment, with protocols likely to apply to shallow tropical lentic systems elsewhere.

KEY WORDS: Artificial substrate · Biomonitoring · Colonisation · Freshwater · Macroinvertebrate · Tropical · Urban reservoir · Singapore

INTRODUCTION

The importance of biological monitoring (biomonitoring) of water quality is recognised in many countries worldwide (Spellerberg 1991, Barbour et al. 1996). The resident biota of aquatic systems indicate the quality of their environments because they are sensitive to the changes or stresses which occur within them (Bis et al. 2000, Shostell & Williams 2007). As a tool in ecological monitoring, biomonitoring can reveal both short-term and cumulative effects (Rosenberg & Resh 1993), effectively complementing chemical monitoring procedures that may miss sporadic or unpredictable events (Spellerberg 1991).

Benthic macroinvertebrates are the most commonly used group of freshwater organisms for the biological assessment of water quality (Wiederholm 1980, Rosenberg & Resh 1993, Thorne & Williams 1997) because they offer many advantages as continuous biological indicators of the waters they inhabit. They are ubiquitous, diverse, abundant and represent a wide range of pollution sensitivity (Cairns & Dickson 1971, Modde & Drews 1990, Battegazzore et al. 1994). Their relatively long life cycle and sedentary nature makes them ideal organisms for spatial and temporal monitoring of the aquatic environment (Rosenberg & Resh 1993). In addition, sampling equipment is usually low in cost and relatively easy to use. As a concept, biomonitoring

*Corresponding author. Email: dbspat@nus.edu.sg

is well established, and many countries have adopted biotic indices in rapid assessment of streams and rivers during the past 30 yr (Sládeček 1973, Clews & Ormerod 2009). However, biotic indices for lentic environments have been developed only recently in the USA and Europe (Blocksom et al. 2002, Verneaux et al. 2004, Rossaro et al. 2007), and none are available for tropical lake systems in Southeast Asia, although biotic indices for lotic systems have been developed recently for Thailand and Vietnam (Mustow 2002, Nguyen et al. 2004).

As community data form the core of many biomonitoring programmes, accurate and precise quantitative sampling methods (maintaining cost efficiency) are important. Standardisation reduces bias and inter-replicate variability, thus increasing confidence in comparisons among sites sampled (Rosenberg & Resh 1982, Lamberti & Resh 1985, Rinella & Feminella 2005). To date, there is no single sampling device or method for collecting macroinvertebrates that can be applied to all freshwater environments. Instead, a variety of methods have been described for both lotic and lentic habitats (Merritt & Cummins 1996). While standard procedures for sampling benthic macroinvertebrates in lotic habitats are well established, such as kick- or Surber-sampling, these are reliant on the water flow characteristic of streams and rivers and are therefore unsuitable for still waters such as ponds, lakes and reservoirs (Mason 2002). A survey by Rosenberg & Resh (1993) revealed that dredges, grabs and corers were the most common choice for sampling lentic systems, accounting for more than 75% of all the samplers used in a survey of 50 contemporary lentic field studies. While such devices may work in habitats of unconsolidated substrate, they are not always effective in hard-bottom water bodies, such as municipal reservoirs (Downing & Rigler 1984). Artificial substrate samplers or air lifts may be appropriate for the rocky habitats common in urban systems (Voshell & Simmons 1977, Downing & Rigler 1984, Rosenberg & Resh 1993).

Artificial substrates, i.e. some form of standardised material introduced into the water body for a fixed period of time, can have advantages over 'instantaneous' samples. For instance, as benthic macroinvertebrates tend to selectively colonise a substrate, in a heterogeneous environment it can be difficult to ensure that samples collected using grabs, nets, etc. are comparable across sites (Crossman & Cairns 1974). The use of an artificial substrate circumvents this problem by providing a uniform and reproducible area for colonisation by macroinvertebrates (Mason et al. 1973). Artificial substrate samplers are also relatively low in cost, easy to use and can be applied in both lentic and lotic habitats (Czerniawska-Kusza 2004). The primary drawback is that they can only sample what colonises them, and this might not represent the complete macroinvertebrate community (Modde & Drews 1990). Since the 1930s, a variety of artificial substrate designs, including concrete slabs, limestone or rock-filled baskets, and multi-plate Hester-Dendy samplers, have been implemented in both lentic and lotic systems (e.g. Britt 1955, Hester & Dendy 1962, Mason et al. 1967, Kreis et al. 1971, Voshell & Simmons 1977).

Hydraulic, air-lift or other suction devices are less commonly used in quantitative studies and biomonitoring programmes, perhaps because they were developed later and/or need more bulky and expensive equipment (Pearson et al. 1973, van Arkel & Mulder 1975). Many early air-lift devices work like corers, i.e. they required some insertion of the device into the substrate (e.g. Mackey 1972, Larsen 1974), and do not function as well on hard-engineered bottoms. Nevertheless, they are potentially effective in collecting macroinvertebrate samples from natural habitats (Downing & Rigler 1984).

To date, no studies have tested the performance of artificial samplers or suction devices in Southeast Asian lentic systems, including the reservoirs of Singapore. Singapore is a highly urbanised city-state located just south of Peninsula Malaysia, 1°15′ north of the equator. The country's standing waters primarily comprise 15 municipal reservoirs, 14 of which were built within the last century.

The objective of this study was to establish a sampling device applicable to these engineered, hard-bottom lakes. Initially, we conducted a pilot study to identify: (1) a material that encourages macroinvertebrate colonisation and (2) a suitable colonisation period for artificial substrate samplers. In the main study, we trialled 7 novel and classic artificial substrate samplers, and 2 hydraulic suction devices, to establish an appropriate sampling device for surveying macroinvertebrates in the littoral habitats of tropical urban reservoirs. We examined the family diversity, richness, abundance and community structure of invertebrates captured by the 9 different sampling devices. The performance of the samplers was also compared between 2 littoral habitat types (rocky and vegetated). This project is part of a larger endeavour to develop a biotic index for Singapore's lentic environment; however, the sampling protocols should be applicable to shallow tropical lentic systems throughout Southeast Asia.

MATERIALS AND METHODS

Study site. Singapore has a tropical climate; it is warm and wet all year round with relatively uniform temperatures and 2 monsoon periods. The northeast monsoon occurs around November to February, and

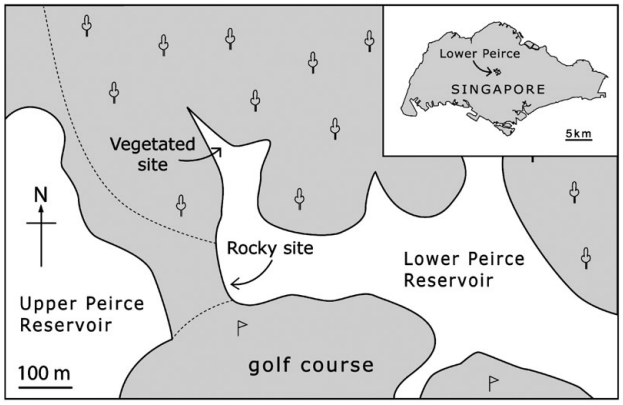

Fig. 1. Lower Peirce reservoir within the Central Catchment Nature Reserve of Singapore. Macroinvertebrate samplers were trialled in 'vegetated' and 'rocky' littoral sites

the southwest monsoon between June and August. Singapore is small (land area ~697 km^2) and very densely populated, hence it is considered among the most water-stressed countries in the world (Tan et al. 2007).

The study was conducted at Lower Peirce Reservoir (1° 22′ 10″ N, 103° 49′ 24″ E), 1 of 4 reservoirs within the Central Catchment Nature Reserve of Singapore (Fig. 1). Constructed in 1900, Lower Peirce is the second oldest reservoir in Singapore (Wee & Corlett 1986). It has a surface area of 59 ha, approximate depth of 7.6 m and a catchment area of 418 ha (Public Utilities Board unpubl. data). It overlies a range of plutonic rock known as Bukit Timah granite, which is typical of central Singapore (Gupta & Pitts 1993, Lu et al. 2005). Adjacent riparian land uses include a golf course (Island Golf Course) to the southeast, a public park (Lower Peirce Reservoir Park) to the east and secondary dipterocarp forests in the remaining areas (Chou et al. 2006).

Macroinvertebrates were sampled from rocky and vegetated sites in the littoral zone of the reservoir (Fig. 1). Bottom substrate composition at the rocky site was dominated by uneven granite boulders, rocks, pebbles and sand, while the vegetated site substrate comprised silt, sand and detritus. At the rocky site, bank-side vegetation was predominantly grass, whereas forest surrounded the banks of the vegetated site.

Pilot study: Comparison of colonisation period and materials used for cage samplers. ***Study design:*** A pilot study was first conducted from March to April 2008 to ascertain which material would be appropriate for artificial substrate samplers as well as to identify a suitable time period for colonisation of the samplers by invertebrates. Cage-type samplers containing 5 different materials were compared in a replicated study design; 3 replicate cages containing each material were deployed at 1.2 m water depth directly on the benthos for 4 wk in rocky and vegetated littoral sites. An identical set of samplers was deployed at the same sites for 6 wk in order to explore the effect of an extended colonisation period on the invertebrate fauna captured by the samplers.

Sampler designs: Cage samplers were constructed from stainless steel wire-mesh (∅ 20 cm; height 10 cm, 1.2 cm^2 mesh size; Fig. 2a,b). The samplers contained either: (1) aquarium balls, (2) plastic bottle brushes, (3) calcium carbonate tubes, (4) coconut brushes and split palm fronds or (5) granite gravel. The amount of each material in the cages was standardised within sampler types: 32 plastic aquarium balls of 4 cm diameter which were constructed of a matrix of criss-crossed plates (commonly used for biofiltration in aquaria); plastic bottle brushes: total length 120 cm and diameter 3 cm; 990 g calcium carbonate tubes with a wall thickness of 3 mm, 3 cm long and with an outer diameter of 1 cm; 2 kg of granite gravel (enough to fill the cage to 4 cm depth); coconut brushes totalling 42 cm long and 4.2 cm diameter plus 90 split palm fronds, each 90 cm in length. The split palm fronds were wound around the interior of the circular cage to form a 'nest' into which the coconut brushes were inserted.

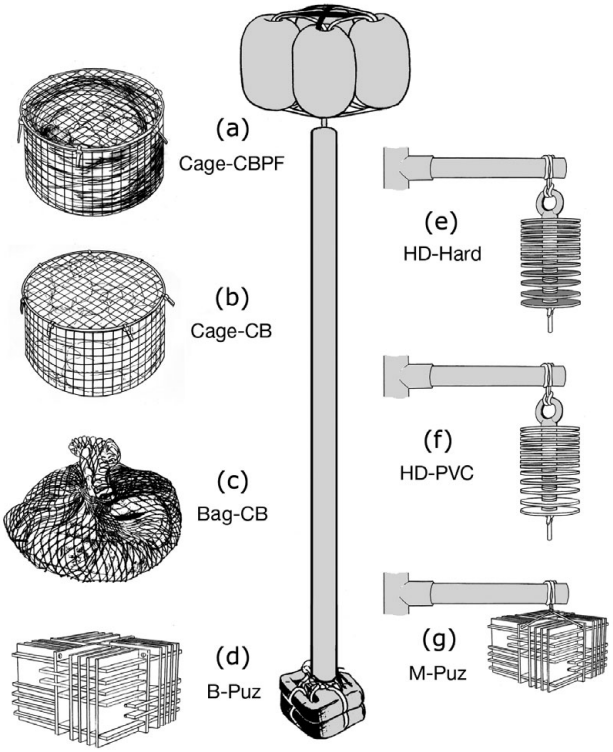

Fig. 2. Framework used to support artificial substrate samplers. The samplers on the left were placed on the benthos and those on the right were suspended in mid-water. (a) Cage with coconut brushes and split palm fronds, (b) cage with coconut brushes only, (c) bag with coconut brushes only, (d) benthic PVC puzzle block, (e) hardboard Hester-Dendy, (f) PVC Hester-Dendy and (g) mid-water PVC puzzle block

Samplers were anchored to the benthos with silicone-covered 0.9 kg lead diving weights. Buoys were secured to the sampler with nylon rope threaded through a PVC pipe (length 70 cm, ∅ 2.4 cm) to aid in deployment and retrieval (Fig. 2).

Processing of invertebrate samples: Samplers were retrieved with a 250 µm mesh, 30 cm frame net (Rinella & Feminella 2005). Entire samplers were then placed in plastic containers filled with soda water to narcotise the invertebrates. They were subsequently transported to the laboratory where the soda water was replaced with 95% methylated ethanol.

The artificial substrate was later washed through a 250 µm sieve. Material removed from the substrate was again preserved in 95% ethanol. Invertebrates were sorted first to order level in a 40 × 25 cm white tray under a good light (Mason 2002), and subsequently to family level (except for Oligochaeta, Hirudinea, Acarina and Diptera pupae) using a low power light microscope (Meiji© EMZ-5). Primary taxonomic references used were Yule & Yong (2004), Dudgeon (1999) and MRC (2008).

Main study: comparison of sampling devices. ***Study design:*** Whereas in the pilot study we compared artificial substrate materials, in the main study we focused more on the sampling device. The material used for the cage type samplers (coconut brushes and split palm fronds) and the colonisation period (4 wk) were determined from the pilot study (see Results).

A total of 4 replicates of 7 artificial substrate samplers were deployed in July 2008 at the rocky and vegetated sites at a depth of 1.2 m at 2 m intervals along a transect which ran parallel to the shore (~5 to 6 m from the bank). The positions of the sampler types were randomised within 4 replicate blocks. These were left in the water for 4 wk to allow colonisation by macroinvertebrates. Sampling using 2 suction devices was carried out on 16 July 2008 (2 wk after the deployment of artificial samplers) along the same transect. Again, 4 replicate samples were collected from rocky and vegetated sites using each device.

Sampler designs: The 9 sampling devices tested in this study comprised 7 artificial substrate samplers and 2 hydraulic suction samplers; 4 of the artificial substrate samplers were placed directly on the bottom and 3 were suspended in mid-water (0.6 m from the bottom, Fig. 2). All sampling devices were novel designs except for the Hester-Dendy constructed from tempered hardboard (Mamola 2005). As in the pilot study, samplers were anchored with diving weights, and buoys were secured with nylon rope threaded through a PVC pipe. Mid-water samplers were suspended by a 3 cm nylon rope off a horizontal arm fixed to the PVC pipe (vertical length 70 cm × horizontal length 36 cm, ∅ 2.4 cm, Fig. 2). Coconut brushes and split palm fronds were identified from the pilot study as the most appropriate materials for cage samplers, so they were further tested in this study (Fig. 2a). Cages which contained only coconut brushes (brush length 112 cm, ∅ 4.2 cm, Fig. 2b) were also trialled. In addition, a bag sampler was designed to increase the surface area of contact between the sampler and the benthos. Here, the cage was replaced by a flexible nylon mesh bag (base diameter range 20–25 cm × height range 15–20 cm, Fig. 2c). The bag contained the same amount of coconut brushes as Cage-CB.

A 3-dimensional PVC 'puzzle' was constructed from sections laser-cut from roughened PVC sheet (0.3 cm thick). The sampler (12 × 12 × 8 cm) comprised 31 flat sections that were interlocked in such a way that 64 compartments of varying sizes were produced (Fig. 2d,g). The puzzle pieces were designed to be easily dismantled for processing. They were trialled on the benthos and in mid-water.

Two types of multiplate Hester-Dendy samplers (Hester & Dendy 1962) were trialled in mid-water, 1 made of tempered hardboard, the other made from roughened PVC. Both Hester-Dendy samplers consisted of 14 circular plates 0.3 cm thick with a diameter of 7.6 cm, designed according to the US Environmental Protection Agency (EPA) and USA Geological Society of America (GSA) standard operating procedures in benthological studies (Mamola 2005; Fig. 2e,f).

The 2 hydraulic sampling devices were designed for rapid sampling of benthic macroinvertebrates. For both devices, a high-walled aluminium quadrat (50 × 50 cm, height 20 cm) fixed to a 1.8 m handle was used to demarcate the sampling area. The suction end of the sampler was then swept within the quadrat for 30 s.

The air-lift sampler was powered by a SCUBA tank and designed to lift bottom fauna and fine sediments but not heavier pebbles and rocks. The device functioned by sucking the top sediments within the quadrat, in contrast to previous air-lift designs which require some insertion of the device into the substrate (e.g. Mackey 1972, Larsen 1974). Suctioned material was discharged into a detachable bag with a mesh size of 250 µm (Fig. 3a).

A water pump sampler constructed from a modified 22 cc high torque 2-stroke single cylinder gasoline engine (Tanaka® Model: TCP-25B/210) was used to sample within the quadrat for 30 s. Benthic material was filtered through a 500 µm mesh within a PVC collection chamber (27 cm length, 10 cm internal diameter, Fig. 3b).

Invertebrate samples were processed following the same protocols used in the pilot study.

Statistical analyses. ***Data treatment:*** Diversity, family richness and total abundance were used to summarise community data (Rosenberg & Resh 1993, Clarke & Warwick 2001). The Shannon-Wiener diversity index (H′)

was used, as it is not strongly influenced by rare taxa (Pires et al. 2000). Bonferroni corrections were applied for multiple tests (Tabachnick & Fidell 2001).

Pilot study: Colonisation Period and materials used for cage samplers: A 3-way repeated measures ANOVA was used to examine the variation observed in diversity, family richness and total abundance of invertebrates between Colonisation Periods (within subjects), between Sites and among Samplers (between subjects). Interactions between Colonisation Period and Site, and Colonisation Period and Sampler type as well as Coloniosation Period , Site and Sampler were also investigated.

Variation in the total abundance, family diversity and richness of invertebrates between Sites and among Samplers was tested further for each Colonisation Period, 4 and 6 wk, respectively, using a 2-way multivariate ANOVA (MANOVA). Where differences were evident between Sites or Samplers, they were examined by 1-way MANOVA and Tukey pairwise comparisons.

Diversity and log-transformed abundance data conformed to the assumptions of ANOVA, and therefore $p < 0.05$ was considered significant. It was not possible to homogenise the variance of the richness of families because there was no variance in richness for 1 treatment; 12 taxa were present in each of the 3 coconut brush samplers retrieved after 4 wk from the rocky site. Therefore, only a more stringent $p < 0.01$ was considered significant for richness (Tabachnick & Fidell 2001).

Main study: comparison of sampling devices: Variation in diversity, richness and abundance among Sites and Samplers was examined using a 2-way MANOVA. Tukey pairwise tests were used to make *post hoc* comparisons. Any interaction between Site and Sampler type for each variable were further investigated using 1-way ANOVA. Due to very low abundances and diversity in the samples collected by both of the hydraulic suction samplers, these data did not fulfil the assumptions of MANOVA and therefore were excluded from all analyses. All data were square-root transformed to homogenise variances and achieve normality (Hair et al. 1995). MANOVA and ANOVA were performed in SPSS for Windows (version 17.0).

Variation in the structure of macroinvertebrate communities (across Samplers and Sites) was examined using non-metric multidimensional scaling (MDS) in PRIMER (Plymouth Routines in Multivariate Ecological Research, version 6) as it is a robust ordination method for analysing community data (Kenkel & Orloci 1986, McCune & Grace 2002, Clarke & Gorley 2006). MDS ordination was based on Bray-Curtis similarities calculated on square-root transformed abundance data (Clarke & Warwick 2001). Differences in community structure between the cluster groups at the 55% similarity level were assessed using a 1-way analysis of similarities (ANOSIM). Pairwise R statistics were used to further investigate variation in communities among clusters. To examine which taxa contributed most to the dissimilarities between clusters, and the similarities within each cluster, a 'similarity percentages' routine (SIMPER) was performed (Clarke & Gorley 2006).

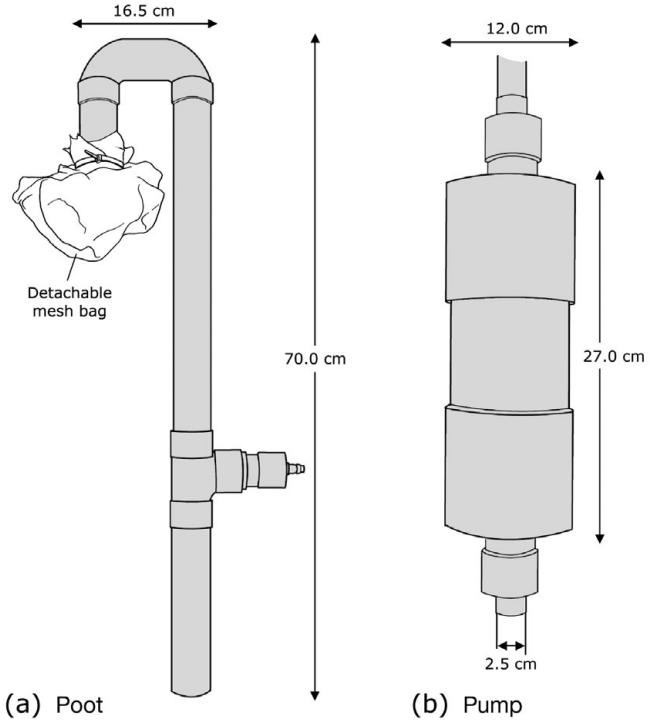

Fig. 3. Hydraulic sampling devices used for collecting an instantaneous sample of invertebrates from the benthos. (a) SCUBA tank-powered air-lift suction sampler, (b) modified gasoline engine water pump sampler

RESULTS

Pilot study: colonisation period and materials used for cage samplers

Between the 2 colonisation periods (4 and 6 wk), there was no significant difference in the family diversity or richness of invertebrates (Table 1). The same was also true of their total abundance after Bonferroni correction for multiple tests (Table 1).

Invertebrate metrics were similar in the 2 sites, viz. rocky and vegetated (Table 2). When the diversity of all sampler types was pooled, a significantly greater diversity of families was evident in samplers retrieved from vegetated site compared to those collected from the rocky site (Table 1). However, when each sampler type was considered separately, this was only significant for the bottle brush (BB) samplers collected from the vegetated site after 4 wk

Table 1. Variation in the family richness, diversity and total abundance of invertebrates between Colonisation Periods (within subjects), between Sites and among Samplers (between subjects; repeated measures ANOVA). Significant differences in diversity and abundance were assumed at $^{*}p < 0.05$ ($^{*}p < 0.01$ for richness)

	df	MS	*F*	p
Abundance				
Within subjects				
Colonisation Period (CP)	1	6.655	6.655	0.018[a]
CP × Sampler	4	1.046	1.046	0.409
CP × Site	1	0.558	0.558	0.464
CP × Sampler × Site	4	1.105	1.105	0.382
Between subjects				
Sampler	4	0.923	15.619	<0.001*
Site	1	0.115	1.939	0.179
Sampler × Site	1	0.138	2.343	0.090
Diversity				
Within subjects				
CP	1	0.540	0.682	0.419
CP × Sampler	4	0.117	1.437	0.248
CP × Site	1	0.914	11.53	0.003*
CP × Sampler × Site	4	1.032	3.258	0.033
Between subjects				
Sampler	4	0.132	0.945	0.459
Site	1	1.326	9.503	0.006*
Sampler × Site	1	0.072	0.514	0.726
Richness				
Within subjects				
CP	1	0.067	0.011	0.917
CP × Sampler	4	2.525	0.424	0.789
CP × Site	1	4.267	0.717	0.407
CP × Sampler × Site	4	5.142	0.864	0.502
Between subjects				
Sampler	4	28.958	4.683	0.008*
Site	1	38.400	6.210	0.022
Sampler × Site	1	7.608	1.230	0.330

[a]Not significant after Bonferroni correction for multiple tests

Table 2. Results of a 1-way multivariate ANOVA to test for among-sampler differences in family diversity, richness and total abundance of macroinvertebrates. $^{*}p < 0.05$

Colonisation period	Site	Variable	df	MS	*F*	p
4 wk	Vegetated	Diversity	4	0.135	1.451	0.288
		Richness	4	3.433	1.073	0.420
		Abundance	4	0.173	4.571	0.023*
	Rocky	Diversity	4	0.116	0.779	0.564
		Richness	4	18.900	2.268	0.134
		Abundance	4	0.599	7.668	0.004*
6 wk	Vegetated	Diversity	4	0.245	2.028	0.166
		Richness	4	9.733	1.304	0.333
		Abundance	4	0.113	1.380	0.308
	Rocky	Diversity	4	0.079	0.948	0.480
		Richness	4	11.690	1.998	0.179
		Abundance	4	0.286	10.406	0.002*

(df = 1, F = 11.255, p = 0.028) and aquarium balls (AB) retrieved after 6 wk (df = 1, F = 45.791; p = 0.002). This was reflected also by an interaction between colonisation periods and sites (Table 1). Other samplers yielded a similar diversity of invertebrates from both sites.

The abundance of invertebrates differed significantly among the 5 samplers (Table 2, Fig. 4) with the coconut brush and split palm frond (CBPF) samplers yielding the highest abundance (Table 1, Fig. 4, Tukey pairwise test, p < 0.001). When considered separately for each colonisation period and site, the abundance of animals within the CBPF was significantly higher than all other samplers after 6 wk in the rocky site, greater than in the AB, ceramic tubes (CT), and granite chips (G) after 4 wk in the rocky site, and greater than in the BB and CT samplers after 4 wk in the vegetated site (Tukey pairwise test, p < 0.05; Table 2).

Overall, the richness of invertebrates collected CBPF samplers was higher than in CT and G samplers (Tukey pairwise test, p < 0.05, Fig. 4). The diversity of invertebrates did not differ significantly among samplers.

Main study: comparison of sampling devices

In total, 26 099 specimens were collected across all 54 samples from both site types. These represented 19 macroinvertebrate families plus 3 other taxa assessed at higher taxonomic levels (Acarina, Oligochaeta and Diptera pupae; Table 3).

Diversity, family richness and total abundance differed significantly among all 7 artificial substrate samplers but not between sites (Table 4, Figs. 5–7). A significant interaction was found for the diversity of families between site and sampler type (df = 6, F = 3.390, p = 0.012), reflecting a higher diversity of invertebrates sampled by the cage-CBPF in the vegetated site compared to the rocky site (df = 1, F = 9.688, p = 0.036).

The CBPF sampler captured a significantly greater abundance of taxa than all other samplers (Fig. 5). In general, benthic artificial substrate samplers containing coconut brushes with or without split palm fronds (i.e. Cage-CBPF, Cage-CB and Bag-CB) yielded a significantly greater richness, diversity and total abundance of invertebrate families than those without (Tukey pair-wise test, p < 0.05, Figs. 5–7). Only the richness of families captured by the benthic PVC puzzle (B-Puz) sampler

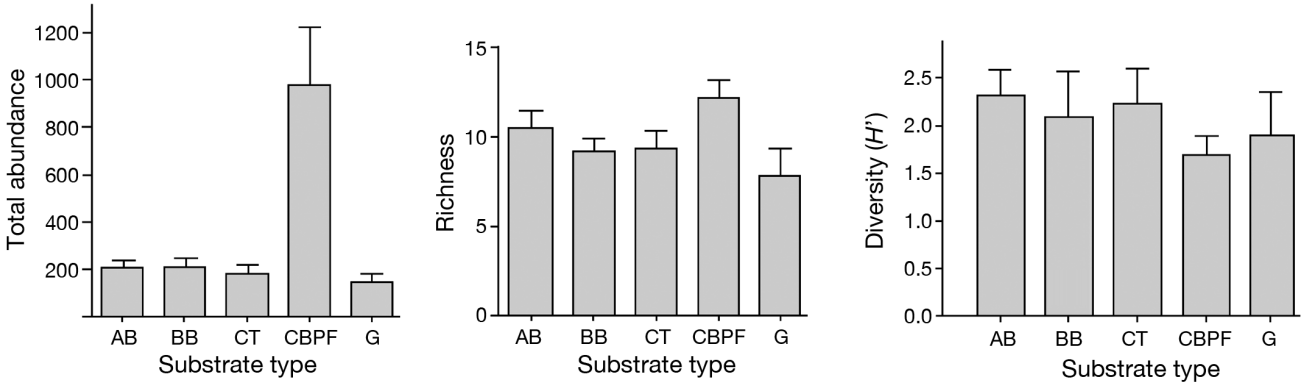

Fig. 4. Variation in total abundance, family richness and diversity (+ SE) of benthic macroinvertebrates that colonised each of 5 artificial substrate cage samplers (data pooled for both colonisation periods and sites). Coconut brushes with split palm frond samplers yielded a significantly greater abundance ($p < 0.001$). AB: aquarium balls; BB: bottle brushes; CT: ceramic tubes; CBPF: cococut brushes with palm fronds; G: granite

Table 3. Macroinvertebrate taxa collected and identified from experimental studies in Lower Peirce reservoir, Singapore. Presence (+) and absence (–) of each taxon captured by each sampler are indicated. See Table 5 for sampler abbreviations

Taxon	Cage-CBPF	Cage–CB	Bag–CB	B–Puz	M–Puz	HD–Hard	HD–PVC	Poot	Pump
Palaemonidae	+	+	+	+	+	+	+	+	–
Viviparidae	+	+	+	+	+	–	+	+	–
Ampullaridae	+	+	+	+	–	–	–	+	–
Thiaridae	+	–	–	–	–	–	–	–	–
Lymnaeidae	+	–	–	–	–	–	–	–	–
Ancycidae	+	–	–	–	–	–	–	–	–
Planorbidae	+	+	–	+	–	–	–	–	–
Chironomid larvae	+	+	+	+	+	+	+	+	+
Diptera pupae	+	+	+	+	+	+	+	+	+
Ecnomidae	+	+	+	+	+	–	+	–	–
Polycentropodidae	+	+	+	+	–	–	+	+	–
Leptoceridae	+	+	+	+	–	–	–	–	–
Hydroptilidae	+	–	+	–	–	–	–	–	–
Polymitarcyidae	+	+	+	+	+	+	–	–	–
Caenidae	+	+	+	+	–	–	–	+	–
Baetidae	+	+	+	+	+	–	–	–	–
Coenagrionidae	+	+	+	–	–	+	–	+	–
Libellulidae	+	+	+	+	–	–	–	+	–
Micronectidae	+	+	+	+	+	+	+	+	–
Acarina	–	+	+	–	–	–	–	+	+
Oligochaeta	+	+	+	–	–	–	–	+	–

was similar to that sampled by Bag-CB and Cage-CB, Fig. 6.

MDS ordination of community data revealed 4 main clusters at the 55% similarity level with a stress value of 0.08 (Fig. 8), which indicates a good ordination with minimal risk of misinterpretation (Clarke & Warwick 2001). Samplers within each cluster are summarised in Table 5. Invertebrate communities varied significantly among the 4 clusters (ANOSIM, global $R = 0.896$, $p = 0.001$, Table 6). The families that contributed more than 10% to the dissimilarities among the 4 main clusters were Chironomidae (the main driver for differences between all pairs of clusters) as well as Polymitarcyidae and Micronectidae (Table 6).

Table 4. Variation in family diversity, richness and total abundance of invertebrates between sites and among samplers (2-way multivariate analysis of variance; *$p < 0.05$). See Figs. 5–7 for pairwise comparisons between sampler types

Factor	Variable	df	MS	*F*	p
Sampler	Diversity	6	0.681	77.795	<0.001*
	Richness	6	2.414	26.992	<0.001*
	Abundance	6	0.780	97.028	<0.001*
Site	Diversity	1	0.015	1.743	0.116
	Richness	1	0.105	1.172	0.979
	Abundance	1	0.000	0.041	0.633
Sampler × Site	Diversity	6	0.030	3.390	0.037*
	Richness	6	0.049	0.546	0.305
	Abundance	6	0.016	2.028	0.117

DISCUSSION

This study represents the first time that artificial substrate and hydraulic suction devices have been tested in the tropics. From the pilot study, we established that a colonisation period of 4 wk was sufficient to capture key groups of invertebrate taxa since a prolonged colonisation period of 6 wk yielded no additional richness or diversity of invertebrate families. We also determined that, among the 5 substrate types initially tested, CBPF samplers attained the greatest total abundance of all macroinvertebrate taxa, including those which are sensitive to disturbance and therefore important for biomonitoring purposes, such as Ephe-

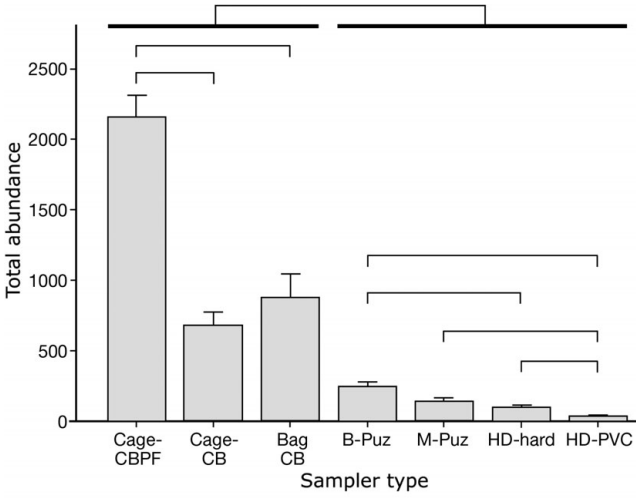

Fig. 5. Variation in mean total abundance (+ SE) of macroinvertebrates that colonised each of 7 artificial substrate samplers after 4 wk. Fine horizontal connecting bars indicate significant differences (Tukey pairwise tests; $p < 0.05$). Invertebrate abundance in the samplers under the heavy horizontal bar on the left was significantly greater than in the samplers under the heavy horizontal bar on the right (Tukey pairwise tests; $p < 0.05$). See Table 5 for abbreviations

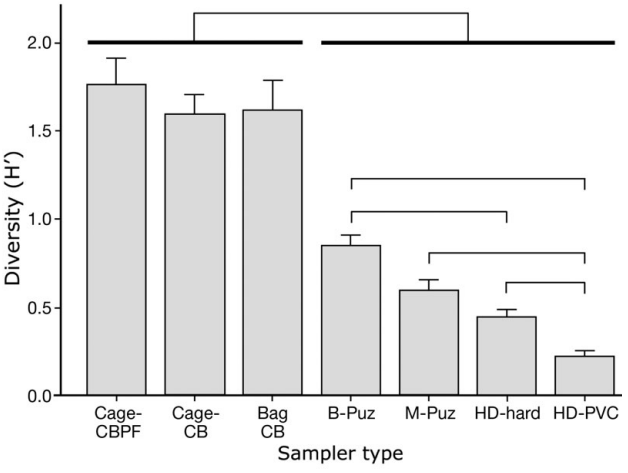

Fig. 7. Variation in mean Shannon-Wiener diversity score (+ SE) captured by each of 7 artificial substrate samplers after 4 wk. Fine horizontal connecting bars indicate significant differences in diversity (Tukey pairwise tests; $p < 0.05$). The diversity of invertebrates captured by samplers under the heavy horizontal bar on the left differed significantly from the diversity within samplers under the heavy horizontal bar on the right (Tukey pairwise tests; $p < 0.05$). See Table 5 for abbreviations

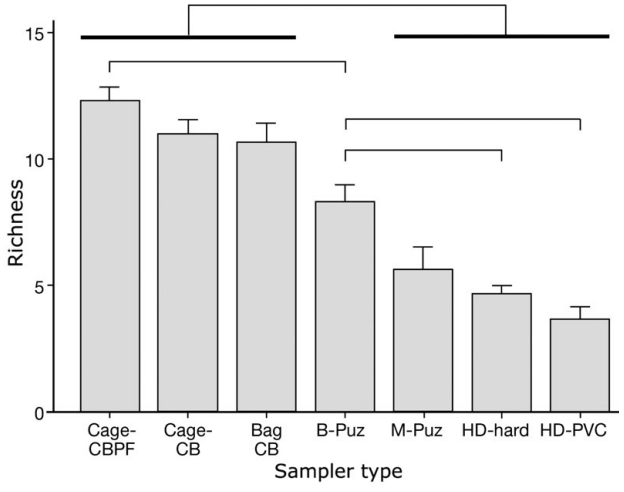

Fig. 6. Variation in mean (+ SE) richness of invertebrate families captured by each of 7 artificial substrate samplers after 4 wk. Fine horizontal connecting bars indicate significant differences in richness (Tukey pairwise tests; $p < 0.05$). Richness of invertebrate families captured by the samplers under the heavy horizontal bar on the left was greater than in the samplers under the heavy horizontal bar on the right (Tukey pairwise tests; $p < 0.05$). See Table 5 for abbreviations

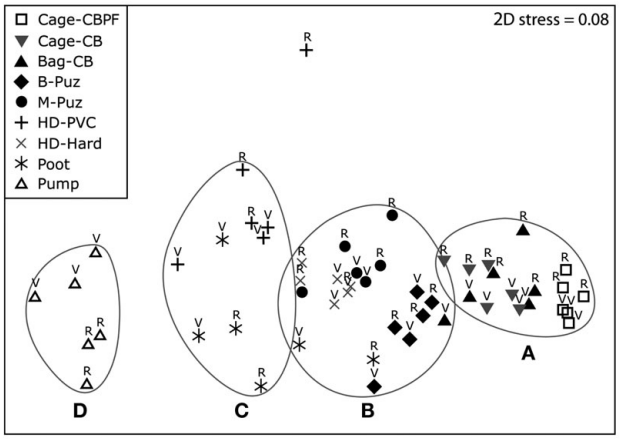

Fig. 8. Two-dimensional multidimensional scaling (MDS) of invertebrates captured using 9 different sampler designs (see Table 5 for descriptions of samplers) at a rocky (R) and a vegetated (V) site. Bray-Curtis similarities were calculated on square-root transformed taxa abundances. Four main clusters (labelled A–D) were identified at the 55% similarity level. The outlier at the top of the plot is a rocky site HD-PVC sample where the number of taxa captured was extremely low

meroptera. Thus CBPF were used in the investigation (the main study) to test the performance of different sampling devices.

The comparison of taxa collected by the 9 different sampling devices tested in the main study indicated that Cage-CBPF was the most effective sampler, since it accrued the greatest family richness and abundance of benthic macroinvertebrates. In contrast, the hydraulic suction devices collected few individuals compared to the other samplers.

No significant differences in community structure were identified between 'rocky' and 'vegetated' sites; this is a surprising result, as macroinvertebrate communities generally exhibit spatial trends (Kerans et al. 1992). Greater macroinvertebrate diversity, richness and abundance might be expected at the vegetated site, given the presence of emergent plants which should have increased habitat heterogeneity, thus providing a greater variety of shelter and food resources (Spänhoff & Arle 2007, Theel et al. 2008). In Lower Peirce, the weed-cutting regime reduced the in-lake vegetation, leaving a relatively uniform silt/sand benthos with occasional woody debris. This habitat may have afforded invertebrates a less heterogeneous habitat than might be expected in a natural, unmanaged lake with riparian vegetation, thus effectively reducing habitat complexity and its potential to support more diverse invertebrate communities (Taniguchi & Tokeshi 2004).

The diversity, richness and abundance of invertebrates trapped by the Bag-CB and the Cage-CB were similar. This suggests that the coconut brushes were responsible for the colonisation of macroinvertebrates, and not the device within which they were contained. The greater range of taxa and number of individuals collected by the samplers using coconut brushes could be explained by the greater habitat complexity offered by this substrate. Habitat complexity plays a major role in determining benthic community structure (e.g. Thomaz et al. 2008). Theoretically, greater complexity will support greater diversity, richness and abundance of species because complex habitats possess more spatial resources (physical niches) than less complex ones (O'Connor 1991, Kostylev et al. 2005, Shumway et al. 2007). Complex habitats serve as predation refuges for benthic macroinvertebrates (Everett & Ruiz 1993, Taniguchi & Tokeshi 2004), and enhanced substrate heterogeneity should enable more species to coexist (Minshall 1984, Hampton 2004). Coconut brushes provide higher surface complexity, greater surface area and more interstitial spaces relative to artificial substrate samplers made from PVC or hardboard.

Many studies have demonstrated that the presence of woody substrate is positively correlated to invertebrate abundance and diversity (Everett & Ruiz 1993, Scealy et al. 2007). Coconut brushes and split palm fronds are organic and thus likely to be important resources to various macroinvertebrates (Minshall 1984). For example, case- and tube-making larvae of Trichoptera and Chironomidae tend be attracted to woody substrate and use it as a

Table 5. Sampler types were classed into 4 clusters (A to D) by hierarchical agglomerative clustering of invertebrate abundance at the 55% similarity level

	Abbreviation	Area	Sampler type
A	Cage-CBPF	Benthos	Cage with coconut brushes and fronds
	Cage-CB	Benthos	Cage with coconut brushes only
	Bag-CB	Benthos	Bag with coconut brushes only
B	B-Puz	Benthos	Benthic PVC puzzle block
	M-Puz	Mid-water	Mid-water PVC puzzle block
	HD-Hard	Mid-water	Tempered hardboard Hester-Dendy
C	HD-PVC	Mid-water	PVC Hester-Dendy
	Poot	Benthos	Air-lift suction device
D	Pump	Benthos	Modified gasoline engine water pump suction device

Table 6. Pairwise differences in invertebrate family abundance between the cluster groups defined in Table 5 (Global R, with p values in parentheses). Increasing R values represent increasing dissimilarity between the clusters. Families that contributed >10% to dissimilarities are also listed

Clusters (sampler types within each cluster)	B B-Puz, M-Puz, HD-Hard	C HD-PVC	D Poot
A Cage-CBPF, Cage-CB, Bag-CB	R = 0.843 (p < 0.001) Chironomidae (41.16%) Polymitarcyidae (13.14%)	R = 0.999 (p < 0.001) Chironomidae (45.58%) Polymitarcyidae (12.26%)	R = 1.000 (p < 0.001) Chironomidae (48.04%) Polymitarcyidae (11.51%)
B B-Puz, M-Puz, HD-Hard	–	R = 0.753 (p < 0.001) Chironomidae (36.80%)	R = 1.000 (p < 0.001) Chironomidae (49.53%)
C HD-PVC, Poot	–	–	R = 0.861 (p < 0.001) Chironomidae (39.77%) Micronectidae (21.32%)

raw material or as a food source (Hanna 1961, Higler 1975, Brennan et al. 1978). Several taxa such as polymitarcyid and chironomid larvae may have therefore found the coconut brushes and split palm fronds attractive (Hubbard 1984, Dudgeon 1999). Palm fronds which were wound around the cage also appeared to trap sediment, representing another organic substrate that could support additional macroinvertebrate diversity (O'Connor 1991).

The PVC puzzle block samplers (B-Puz and M-Puz) were designed to provide a range of spaces and surfaces to attract fauna, but they were less effective compared to the coconut brush samplers in capturing diversity, richness and numbers of macroinvertebrates. This may have been because the voids were too large in relation to the body size of the macroinvertebrates. PVC (despite being roughened) is perhaps not a satisfactory surface for sedentary macroinvertebrates to cling to (Minshall 1984). As a hard substrate, PVC materials are also impenetrable; thus, burrowing organisms will be deterred (Minshall 1984).

Among all the artificial substrate samplers, the industry standard multiplate Hester-Dendy samplers (Mamola 2005) yielded the fewest macroinvertebrates on all measures. PVC Hester-Dendy samplers supported an even lower diversity and macroinvertebrate abundance than hardboard ones. The hardboard substrate may have performed better due its organic make-up and greater surface rugosity.

In general, the mid-water samplers were ineffective. Being light-weight, small in size (Hester-Dendy samplers) and suspended in the water column means that mid-water samplers are less stable than benthic samplers (Minshall 1984). Stability of the substrate plays an important role in the distribution of macroinvertebrates (Luedtke & Brusven 1976, Malmqvist et al. 1978), with at least an intermediate degree of stability needed to support a more abundant and diverse community (Stanford & Ward 1983). In addition, macroinvertebrates in lentic environments tend to be closely associated with the benthos (Merritt & Cummins 1996). Samplers closer to the bottom substrate are therefore expected to accumulate greater densities of organisms (Rosenberg & Resh 1993).

The poor performance of the 2 hydraulic suction samplers could have been due to disturbance (e.g. sediment resuspension) caused by the samplers themselves (Blomqvist 1991). Since macroinvertebrates often respond to disturbance quickly (Resh & Rosenberg 1984) they may have escaped the range of the suction samplers. In addition, there were probably few animals present within the 0.25 m^2 quadrat. The rocky site and sand/silt benthos of the vegetated littoral sites may have afforded limited suitable habitat and thus support relatively low densities of fauna (Shostell & Williams 2007). The habitat provided by the coconut samplers was potentially more attractive than the natural environment, perhaps explaining their efficacy.

Did the artificial substrate sample a true representation of the actual community assemblage within the reservoir? Certain taxa may selectively colonise artificial substrate due to their specific habitat preference. For example, Polymitarcyidae and some Chironomidae have a preference for woody substrate, so these could be overrepresented in the coconut brush samplers (Minshall 1984, Dudgeon 1999, Hubbard 1984). However, for regular biomonitoring programs, the need for reproducibility is more critical than a full community representation, as comparisons between standardised samples have to be made in order to determine and detect biotic responses to changes in water quality (Rosenberg & Resh 1993, Parsons & Norris 1996, Rinella & Feminella 2005).

Generally, very little is known about the macroinvertebrate communities of tropical lentic systems (Dudgeon 1999, 2000), and this is particularly true for Singapore's reservoirs. Except for a few orders, such as Gastropoda and Decapoda, for which many species have been identified, taxonomic knowledge to the species level of other macroinvertebrate taxa, including Chironomidae, Ephemeroptera, Trichoptera, Odonata, Oligochaeta and Acarina, is limited (Corlett 1992, Ng 1992, Ng et al. 1993, Clements et al. 2006). The absence of regional macroinvertebrate keys for species-level identification eliminated the possibility of finer taxonomic resolution in the present study. Even though identification to higher taxonomic groups may mask or underestimate diversity, family-level biomonitoring should still remain capable of detecting major trends and differences in water quality (Furse et al. 1984, Armitage et al. 1987, Thorne & Williams 1997). This gap in knowledge stresses the urgent need for work on the identification of aquatic macroinvertebrates in Southeast Asia.

In conclusion, the Cage-CBPF sampler was the most effective in terms of capturing the highest abundance and family richness of benthic macroinvertebrates compared to a range of other novel artificial substrate samplers, traditional suction devices and Hester-Dendy samplers. More generally, any sampler containing coconut brushes yielded a greater richness and diversity of invertebrate families than those without. Cage-CBPF samplers performed consistently on both hard- and soft-bottomed littoral substrata, further supporting their suitability as a standardised sampler for biomonitoring among different lentic habitat types. This sampler is easy to fabricate from materials that are cheap and readily available in the tropics, and it therefore has substantial potential to be used for lentic biomonitoring programmes in low-latitude countries around the world.

Acknowledgements. We thank the Public Utilities Board (PUB) and the National Parks Board (Nparks) for permission to access Lower Peirce Reservoir and collect invertebrates. We also thank PUB staff at the Lower Peirce pumping station for provision of boats and assistance in field trials, and the many student assistants who helped us to fabricate samplers and process the samples. This research was supported by grant number R-154-000-280-112.

LITERATURE CITED

Armitage PD, Gunn RJM, Furse MT, Wright JF, Moss D (1987) The use of prediction to assess macroinvertebrate response to river regulation. Hydrobiologia 144:25–32

Barbour MT, Gerritsen J, Griffith GE, Frydenborg R, McCarron E, White JS, Bastian ML (1996) A framework for biological criteria for Florida streams using benthic macroinvertebrates. J N Am Benthol Soc 15:185–211

Battegazzore M, Guzzini A, Pagnotta R (1994) Compared use of macroinvertebrate samplers for the evaluation of water quality in rivers of different sizes. Limnologica 24:43–49

Bis B, Zdanowicz A, Zalewski M (2000) Effects of catchment properties on hydrochemistry, habitat complexity and invertebrate community structure in a lowland river. Hydrobiologia 422-423:369–387

Blocksom KA, Kurtenbach JP, Klemm DJ, Fulk FA, Cormier SM (2002) Development and evaluation of the lake macroinvertebrate integrity index (LMII) for New Jersey lakes and reservoirs. Environ Monit Assess 77:311–333

Blomqvist S (1991) Quantitative sampling of soft-bottom sediments: problems and solutions. Mar Ecol Prog Ser 72: 295–304

Brennan A, McLachlan AJ, Wotton RS (1978) Particulate material and midge larvae (Chironomidae: Diptera) in an upland river. Hydrobiologia 59:67–73

Britt NW (1955) New methods of collecting bottom fauna from shoals or rubble bottom of lakes and streams. Ecology 36:524–525

Cairns J Jr, Dickson KL (1971) A simple method for the biological assessment of the effects of waste discharges on aquatic bottom-dwelling organisms. J Water Pollut Control Fed 43:755–772

Chou LM, Tan HTW, Yeo DCJ (2006) The natural heritage of Singapore. Prentice Hall, Singapore

Clarke RK, Gorley RN (2006) PRIMER v6: user manual/tutorial. PRIMER-E, Plymouth

Clarke RK, Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edn. PRIMER-E, Plymouth

Clements R, Lian PK, Tien ML, Meier R, Li D (2006) Importance of reservoirs for the conservation of freshwater molluscs in a tropical urban landscape. Biol Conserv 128: 136–146

Clews E, Ormerod SJ (2009) Improving bio-diagnostic monitoring using simple combinations of standard biotic indices. River Res Appl 25:348–361

Corlett RT (1992) The ecological transformation of Singapore, 1819–1990. J Biogeogr 19:411–420

Crossman JS, Cairns J Jr (1974) A comparative study between two different artificial substrate samplers and regular sampling techniques. Hydrobiologia 44:517–522

Czerniawska-Kusza I (2004) Use of artificial substrates for sampling benthic macroinvertebrates in the assessment of water quality of large lowland rivers. Pol J Environ Stud 13:579–584

Downing JA, Rigler FH (1984) A manual on methods for the assessment of secondary productivity in freshwaters. Blackwell, UK

Dudgeon D (1999) Tropical Asian streams: zoobenthos, ecology and conservation. Hong Kong University Press, Hong Kong

Dudgeon D (2000) The ecology of tropical Asian rivers and streams in relation to biodiversity conservation. Annu Rev Ecol Syst 31:239–263

Everett RA, Ruiz GM (1993) Coarse woody debris as a refuge from predation in aquatic communities: an experimental test. Oecologia 93:475–486

Furse MT, Moss D, Wright JF, Armitage PD (1984) The influence of seasonal and taxonomic factors on the ordination and classification of running-water sites in Great Britain and on the prediction of their macro-invertebrate communities. Freshw Biol 14:257–280

Gupta A, Pitts J (1993) Physical adjustments in a changing landscape: the Singapore story. Singapore University Press, Singapore

Hair JF Jr, Black WC, Babin BJ, Anderson RE, Tatham RL (1995) Multivariate data analysis, 6th edn. Pearson Education, Upper Saddle River, NJ

Hampton SE (2004) Habitat overlap of enemies: temporal patterns and the role of spatial complexity. Oecologia 138: 475–484

Hanna HM (1961) Selection of materials for case-building by larvae of caddisflies (Trichoptera). Proc R Entomol Soc Lond Ser A Gen Entomol 36:37–47

Hester FE, Dendy JS (1962) A multiple-plate sampler for aquatic macroinvertebrates. Trans Am Fish Soc 91:420–421

Higler LWG (1975) Reactions of some caddis larvae (Trichoptera) to different types of substrate in an experimental stream. Limnol Oceanogr 19:951–957

Hubbard MD (1984) A revision of the genus *Povilla* (Ephemeroptera: Polymitarcyidae). Aquat Insects 6:17–35

Kenkel NC, Orloci L (1986) Applying metric and nonmetric multidimensional scaling to ecological studies: some new results. Ecology 67:919–928

Kerans BL, Karr JR, Ahlstedt SA (1992) Aquatic invertebrate assemblages: spatial and temporal differences among sampling protocols. J N Am Benthol Soc 11:377–390

Kostylev VE, Erlandsson J, Mak YM, Williams GA (2005) The relative importance of habitat complexity and surface area in assessing biodiversity: fractal applications on rocky shores. Ecol Complex 2:272–286

Kreis RD, Smith RL, Moyer JE (1971) The use of limestone filled basket samplers for collecting reservoir macroinvertebrates. Water Res 5:1099–1106

Lamberti GA, Resh VH (1985) Comparability of introduced tiles and natural substrates for sampling lotic bacteria, algae, and macroinvertebrates. Freshw Biol 15:21–30

Larsen PF (1974) A remotely operated shallow water benthic suction sampler. Chesapeake Sci 15:176–178

Lu XX, Wong PP, Chou LM (2005) Singapore's biophysical environment. McGraw-Hill Education (Asia), Singapore

Luedtke RJ, Brusven MA (1976) Effects of sand sedimentation on colonization of stream insects. J Fish Res Board Can 33:1881–1886

Mackey AP (1972) An air-lift for sampling freshwater benthos. Oikos 23:413–415

Malmqvist B, Nilsson LM, Svensson BS (1978) Dynamics of detritus in a small stream in southern Sweden and its influence on the distribution of bottom animal communities. Oikos 31:3–16

Mamola M (2005) Procedure for collecting benthic macroinvertebrates using a Hester-Dendy sampler. Environmental Monitoring Program, Department of Pesticide

Regulation, Sacramento, CA
Mason CF (2002) Biology of freshwater pollution, 4th edn. Prentice Hall, Harlow
Mason WT Jr, Anderson JB, Morrison GE (1967) A limestone-filled artificial substrate sampler-float unit for collecting macroinvertebrates in large streams. Prog Fish-Cult 29:74
Mason WT Jr, Weber CI, Lewis PA, Julian EC (1973) Factors affecting the performance of basket and multiplate macroinvertebrate samplers. Freshw Biol 3:409–436
McCune B, Grace JB (2002) Analysis of ecological communities. MjM Software Design, Gleneden Beach, OR
MRC (Mekong River Commission) (2008) Identification of freshwater invertebrates of the Mekong river and its tributaries. Available at www.mrcmekong.org
Merritt RW, Cummins KW (1996) An introduction to the aquatic insects of North America, 3rd edn. Kendall-Hunt Publishing Company, Dubuque, IA
Minshall GW (1984) Aquatic insect-substratum relationships. In: Resh VH, Rosenberg DM (eds) The ecology of aquatic insects. Praeger, New York, p 358–400
Modde T, Drews HG (1990) Comparison of biotic index values for invertebrate collections from natural and artificial substrates. Freshw Biol 23:171–180
Mustow SE (2002) Biological monitoring of rivers in Thailand: use and adaptation of the BMWP score. Hydrobiologia 479:191–229
Ng PKL (1992) A guide to freshwater life in Singapore, 2nd edn. Singapore Science Centre, Singapore
Ng PKL, Chou LM, Lam TJ (1993) The status and impacts of introduced freshwater animals in Singapore. Biol Conserv 64:19–24
Nguyen XQ, Mai DY, Pinder C, Tilling S (2004) Biological surveillance of freshwaters: a practical manual and identification key for use in Vietnam. Vietnam National University Publishers, Hanoi
O'Connor NA (1991) The effects of habitat complexity on the macroinvertebrates colonising wood substrates in a lowland stream. Oecologia 85:504–512
Parsons M, Norris RH (1996) The effects of habitat specific sampling on biological assessment of water quality using a predictive model. Freshw Biol 36:419–434
Pearson RG, Litterick MR, Jones NV (1973) An air-lift for quantitative sampling of the benthos. Freshw Biol 3: 309–315
Pires AM, Cowx IG, Coelho MM (2000) Benthic macroinvertebrate communities of intermittent streams in the middle reaches of the Guadiana Basin (Portugal). Hydrobiologia 435:167–175
Resh VH, Rosenberg DM (1984) The ecology of aquatic insects. Praeger, New York
Rinella DJ, Feminella JW (2005) Comparison of benthic macroinvertebrates colonising sand, wood, and artificial substrates in a low-gradient stream. J Freshw Ecol 20: 209–220
Rosenberg DM, Resh VH (1982) The use of artificial substrates in the study of freshwater benthic macroinvertebrates. In: Cairns J Jr (ed) Artificial substrates. Ann Arbor Science Publishers, Ann Arbor, MI, p 175–235
Rosenberg DM, Resh VH (1993) Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York
Rossaro B, Marziali L, Cardoso AC, Solimini A, Free G, Giacchini R (2007) A biotic index using benthic macroinvertebrates for Italian lakes. Ecol Indic 7:412–429
Scealy JA, Mika SJ, Boulton AJ (2007) Aquatic macroinvertebrate communities on wood in an Australian lowland river: experimental assessment of the interactions of habitat, substrate complexity and retained organic matter. Mar Freshw Res 58:153–165
Shostell JM, Williams BS (2007) Habitat complexity as a determinate of benthic macroinvertebrate community structure in cypress tree reservoirs. Hydrobiologia 575: 389–399
Shumway CA, Hofmann HA, Dobberfuhl AP (2007) Quantifying habitat complexity in aquatic ecosystems. Freshw Biol 52:1065–1076
Sládeček V (1973) The reality of three British biotic indices. Water Res 7:995–1002
Spänhoff B, Arle J (2007) Setting attainable goals of stream habitat restoration from a macroinvertebrate view. Restor Ecol 15:317–320
Spellerberg IF (1991) Monitoring ecological change. Cambridge University Press, Cambridge
Stanford JA, Ward JV (1983) Insect species diversity as a function of environmental variability and disturbance in stream systems. In: Barnes JR, Minshall GW (eds) Stream ecology: application and testing of general ecological theory. Plenum Press, New York, NY, p 265–278
Tabachnick BG, Fidell LS (2001) Using multivariate statistics. Allyn & Bacon, London
Tan HW, Chou LM, Yeo DCJ, Ng PKL (2007) The natural heritage of Singapore, 2nd edn. Pretence Hall, Singapore
Taniguchi H, Tokeshi M (2004) Effects of habitat complexity on benthic assemblages in a variable environment. Freshw Biol 49:1164–1178
Theel HJ, Dibble ED, Madsen JD (2008) Differential influence of a monotypic and diverse native aquatic plant bed on a macroinvertebrate assemblage; an experimental implication of exotic plant induced habitat. Hydrobiologia 600:77–87
Thomaz SM, Dibble ED, Evengelista LR, Higuti J, Mbini L (2008) Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. Freshw Biol 53:358–367
Thorne RJ, Williams WP (1997) The response of benthic macroinvertebrates to pollution in developing countries: a multimetric system of bioassessment. Freshw Biol 37: 671–686
van Arkel MA, Mulder M (1975) A device for quantitative sampling of benthic organisms in shallow water by means of a flushing technique. Neth J Sea Res 9:365–370
Verneaux V, Verneaux J, Schmitt A, Lovy C, Lambert JC (2004) The Lake Biotic Index (LBI): an applied method for assessing the biological quality of lakes using macrobenthos; the Lake Châlain (French Jura) as an example. Ann Limnol 40:1–9
Voshell JR, Simmons GM Jr (1977) An evaluation of artificial substrates for sampling macrobenthos in reservoirs. Hydrobiologia 53:257–269
Wee YC, Corlett RT (1986) The city and the forest: plant life in urban Singapore. Singapore University Press, Singapore
Wiederholm T (1980) Use of benthos in lake monitoring. J Water Pollut Control Fed 52:537–547
Yule CM, Yong HS (2004) Freshwater invertebrates of the Malaysian region. Academy of Sciences Malaysia, Kuala Lumpur

Editorial responsibility: Hans Heinrich Janssen, Oldendorf/Luhe, Germany

Submitted: February 12, 2010; Accepted: April 26, 2010
Proofs received from author(s): August 6, 2010