CHAPTER 2

THE IMPLEMENTATION OF BIO-INDICATOR MONITORING OF MACROINVERTEBRATES USING SAMPLER METHOD

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2.1 INTRODUCTION

Bio-indicator is widely known as biological indicator useful for monitoring the water quality in the fastest way by implementing the scoring techniques based on the tolerance of influence to the current water quality. The macroinvertebrates are known as one of the freshwater organisms for the biological assessment of the water quality. The living habitat of macroinvertebrates makes a biggest concern over the tolerance of water pollution. They offer many advantages as a continuous biological indicator of the waters they inhabit.

The quality and condition of water stream flow are influenced by human activities such as agriculture and industrialization which can impair the freshwater ecosystem [1]. Benthic macroinvertebrates offer the advantages of quantifying the ecological impacts of urbanization by examining changes in functional feeding guilds along with species and tolerance to pollution [2,3,4]. The functional feeding groups of aquatic macroinvertebrates were categorized by five groups which are predators, collector-gatherers, collector-filterers, shredder and scrappers. Each of the specified groups have their own preferences of water quality to function accordingly to stabilize the freshwater ecosystem and underwater living structure.

2.2 FUEL

Macroinvertebrates were categories in taxonomic groups, family, genus and species in order to clearly specify the component of functionality in the river habitat. In general, macroinvertebrates have significant roles and serve multiple functions in freshwater ecosystem such as shredders, collectors, grazers, and predators. This categorization evolved within freshwater resources in aquatic living structure due to the specialized feeding structures in the freshwater ecosystem as macroinvertebrates is one of the primary consumers in freshwater stream. Macroinvertebrates basically decompose organic matter and also consume each other and other small organisms. The alteration of adjacent vegetation can modify the complex biotic and abiotic processes that direct the function and characteristics of aquatic communities such as macroinvertebrates [5]. The research study conducted in Patagonian streams under different land uses observed that different functional group metrics such as shredders and collectors have a significant difference in the percentage of existence in the study location [6]. The advantages of using functional groups identification approach are adopted to monitor the water quality level. It is involved similarly and performed well as a metric for species-level identification. Basically, the macroinvertebrates were first classified into a group of orders and family. Table 2.1 below shows some of the Order and family of macroinvertebrates found in the river stream with the classification of functional feeding group.

Table 2.1: The functional feeding group [1]

Order	Family	Functional feeding group
Annelida	Hirudinae	Predators
	Oligochaetae	Collector-gatherer
Coleoptera	Gyrinidae	Predator
	Hydrophilidae	Larvae – Predator; Adult – Collector-gatherer
	Helodidae	Scrapper
	Potamonautidae	Shredder
Diptera	Chironomidae	Collector-gatherer
	Dixidae	Collector-gatherer
	Psychodidae	Collector-gatherer
	Simuliidae	Collector-filterer
	Sphaeriidae	Collector-filterer
	Tipuliidae	Shredder
Ephemeroptera	Beatidae	Larvae – Collector-gatherer Adult – Scrapper
	Letophlebidae	Collector-gatherer
Tricoptera	Hydropsychidae	Larvae – Collector-filterer Adult – Scrapper
Hemiptera	Belostomatidae	Predator
	Corixidae	Larvae – Predator Adult – Scrapper
	Gerridae	Predator
	Notonectidae	Predator

Table 2.1: (Continued)

Order	Family	Functional feeding group
Mollusca	Ancylidae	Scrapper
	Physidae	Scrapper
Odonata	Aesnidae	Predator
	Coenagrionoidae	Predator
	Corduliidae	Predator
	Libellulidae	Predator

Bio-indicator which is macroinvertebrates are describe as shredders, collectors, grazers and predators. Shredders are responsible for wandering at the bottom of the stream flow to find the vegetation that has been in the water. It is more attracted to the coarse particulate organic matter such as woody debris, and other riparian sources. In their body segment's part, the tearing mouthparts were used to tear, shred and rip the leaves as they fed themselves. Some organisms such as caddisfly larvae use the shredded leaves pieces to cover their body as a protective casing.

On the other hand, collectors such as beetles usually filter and collect the smaller particles of organic matter that were found in the bottom of the sedimentation. They wander in the bottom part of the stream flow for dead organisms, detritus and other food particles that get lodged between the rocks. Besides, the grazer group of macroinvertebrates usually found on the rocks and woody debris as they were feeding on periphyton, detritus and submerged aquatic plants. Most of the gastropods such as snails were grazer species.

Furthermore, the predators' group can be in larvae stage or adult stage of macroinvertebrates. The macroinvertebrates with predator function eventually swim underwater as beetles do or fly directly above the surface water such as dragonflies.

2.3 METHOD OF MACROINVERTEBRATES SAMPLING

In biological monitoring or biomonitoring, a study at United

Kingdom by [7] has presented the routine statutory biomonitoring which was relied on semi-quantitative and quantitative sampling methods. Qualitative methods are applying the timed kick-net sampling techniques while quantitative methods mainly implement the Surber sampling. The artificial substrate sampler as designed by [8] were referred to and improvised based on the morphological factor for the current sampling location.

5.4.1 KICK-NET SAMPLING

The kick sampling method is used in most countries due to the benefits of cost-effectiveness, and the outcomes of the analysis were consistent and reliable [9; 10]. For this method, researchers commonly specify the net frame size and the mesh size to be a constant variable during the sampling activity. Besides, the method required standardized procedures to be followed in sampling techniques.

5.4.2 SURBER SAMPLER

In general, the Surber sampling techniques are appropriate for creating the nature of habitat for aquatic life with substrate samplers. The implementation of Surber techniques is related to the time of colonization for macroinvertebrates species as their habitat in Surber [8]. There are a variety of macroinvertebrate samplers that were placed inside the water for a certain water depth. Figure 2.1 below shows the possible sampler design for macroinvertebrates sampling. The artificial substrate may include some aquarium balls, plastic bottle brushes, calcium carbonate tubes, coconut brushes and split palm fronds, and granite gravel. The compatibility of the sampler design should be observed during the study period. The river morphology is contributing to one of the factors for analyzing the suitability of the design. Several pilot studies should be conducted to foresee the trends of macroinvertebrates colonization. Furthermore, the sampler design from [8] was improvised based on the morphological factor of study locations.

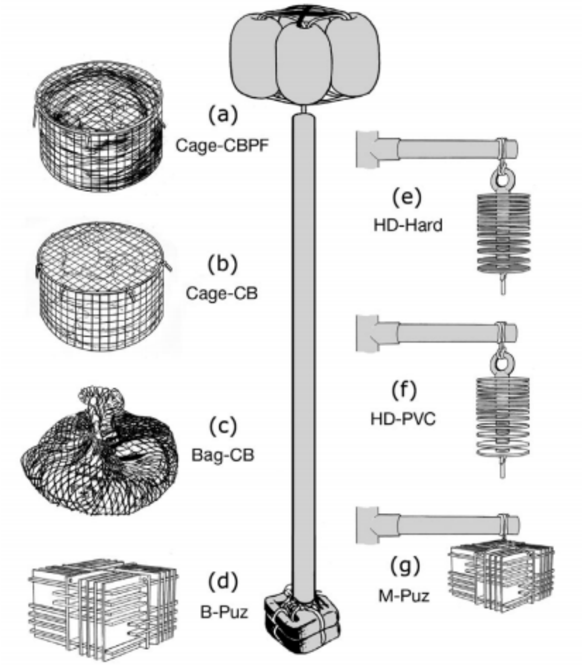

Figure 2.1: Framework used to support artificial substrate samplers [8]

The step-by-step design of the sampler used for collecting the macroinvertebrates sample at Ulu Dengar River were shown in the figure and explanation below. The first step to comes up with the suitable design of sampler for the study location, is to remark on the depth of the water level. The sampler design will be submerged in the water stream and facing towards the water flow. The application of the placement for the sampler is to ensure that the colonization of the macroinvertebrates takes place in the sampler. The dislodged macroinvertebrates from the kick-net sampling techniques is estimated to pass through sampler and then create the colonization for the time being.

The sampler with dimension of 70 cm ~ 40 cm is created using wooden block with 6 replications. Figure 2.2 to Figure 2.11 show the step by step of designing the sampler for macroinvertebrates sampling application.

Figure 2.2: Cut the wooden block according to the specified size

Figure 2.3: Use the L shape fastener to nail the wooden block

The application of quantitative methods with 6 replicates of sampler were placed in the stream flow according to the time specified to monitor the effectiveness and efficiency of sampler to sample macroinvertebrates. Reported by [11], quantitative methods can be more efficient for collecting taxa presents at a site. It may also provide less statistical power for detecting differences in macroinvertebrate assemblages among sites.

Figure 2.4: The complete frame of sampler

Figure 2.5: The six replicates of sampler design

The placement of samplers was conducted at randomly selected location within the riffles of river stream. The sampler was submerged in the flow stream for a period of six days. On the 7th day, the samplers were taken out from the water and the aquatic macroinvertebrates found in the sampler were picked up using plastic pipette, spoon and steel tweezer.

Figure 2.6: Hanson net for internal mesh

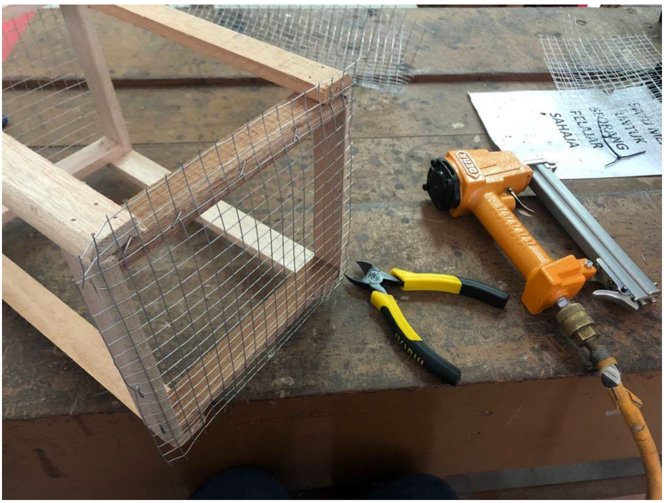

Figure 2.7: Wire mesh as an outer layer

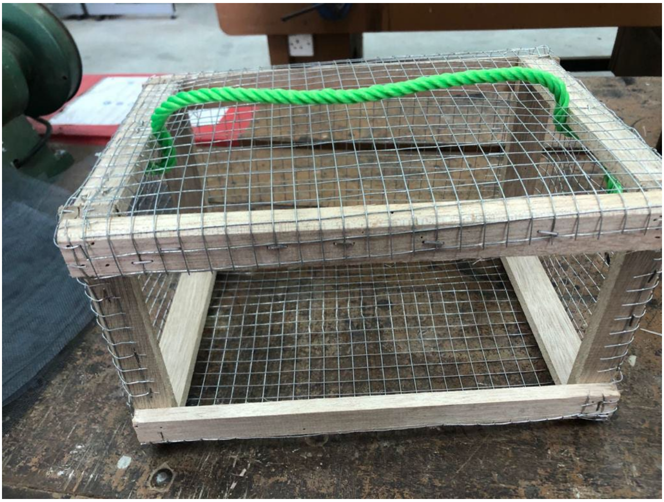

Figure 2.8: Installation of the outer layer for the sampler

Figure 2.9: Tied the handle made up of nylon rope to the sampler

During the collection of macroinvertebrates process, there were three people involved for a period of 30 minutes to 1 hour. The macroinvertebrates were placed in the petri dish for a few minutes for on-site identification process without using any microscope as the macroinvertebrate can also be seen by naked eyes.

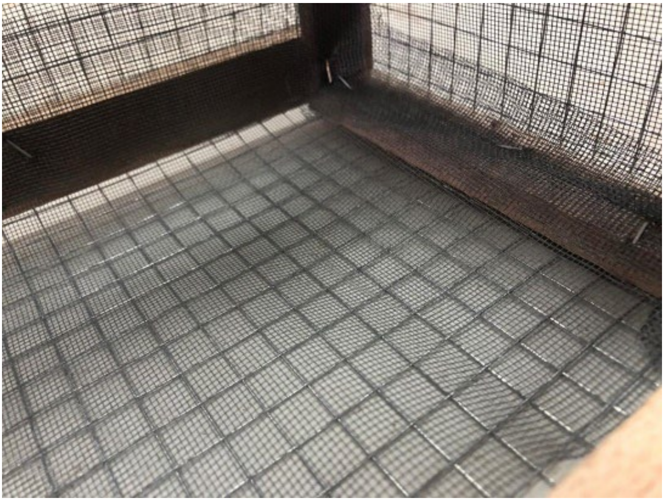

Figure 2.10: Install the wire mesh in the inner part of the sampler

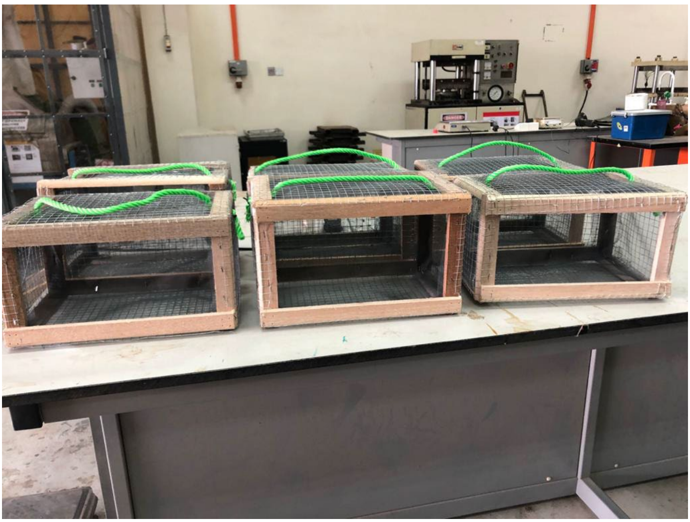

Figure 2.11: The complete six replicates of sampler design

Apparently, reported in the study by [12], there were fewer taxa collected by using the sampler method. The effectiveness of the samplers was different from time to time depending on the seasonal changes and the condition of water flow.

2.4 MACROINVERTEBRATES ABUNDANCE

The abundance of macroinvertebrates using the sampler and kick-net sampling were different due to the time taken to conduct the sampling activity. The limited time of sampling using kick-net and lack of manpower to handle the sampling using kick-net were affecting the outcomes of macroinvertebrates abundance. Compared to the method of sampling using sampler, the application of the sampler on-site required more equipment to maintain the structure of sampler and ensure the safety of the sampler on-site. The crucial part of using sampler as application to monitor the macroinvertebrates assemblages is the need for strong attachment of nylon rope where it is tied to the strong structure around the river stream such as plantation around it.

As the flowrate of river stream could not be set to a standard flowrate and maintain the level of water, the risk of using sampler without strong application of safety might lose the trace of the sampler in the water as it is submerged in the stream flow. Therefore, unexpected seasonal changes or rainy weather influence the accessibility to take out the sampler from the running water. The consistent improvement and back-up sampler need to be planned well to ensure the outcomes of the effectiveness of using sampler as macroinvertebrates monitoring is applicable.

The results in Table 2.2 and Table 2.3 below show some of the lowest species' identification of macroinvertebrates were found at Ulu Dengar River and Gunung Belumut waterfall by using the application of the sampler. Gunung Belumut and Ulu Dengar River are the connected stream flow located in Kluang, Johor. The abundance of macroinvertebrates at Ulu Dengar River is higher compared to the Gunung Belumut waterfall.

Table 2.2: Data tabulation of macroinvertebrates in Ulu Dengar River

	Middle stream Ulu Dengar River						
	Month	May	June	July	11-Aug	18-Aug	4-Sept
No.	Macroinvertebrates Name	Abundance					
1	Mystery snail	3	MCO	MCO	X	x	2
2	Long-mouthed saucer bug	1	MCO	MCO	X	x	x
3	Ramshorn snail	1	MCO	MCO	X	x	x
4	Dragonfly nymphs (long)	3	MCO	MCO	X	x	x
5	Dragonfly nymphs (short)	8	MCO	MCO	4	x	9
6	Whirligig beetle larvae	1	MCO	MCO	8	10	1
7	Cassed caddisfly larvae	1	MCO	MCO	X	x	x
8	Flattened mayfly	3	MCO	MCO	X	x	x
9	Midge pupa	x	MCO	MCO	1	2	x
10	Water beetle	x	MCO	MCO	1	2	1
11	Stonefly nymph	x	MCO	MCO	4	x	x
12	Leeches	x	MCO	MCO	x	5	3
13	Burrowing mayfly	X	MCO	MCO	x	3	9
14	Two-tailled demoiselle	X	MCO	MCO	x	2	12
15	Non-bitting midge larvae	1	MCO	MCO	x	1	2
16	Common dragonfly	X	MCO	MCO	x	6	x
17	Common damselfly	X	MCO	MCO	x	4	1
18	Water penny	X			x	1	1
19	River prawn	X			x	1	x
20	Prong-gillled larvae	X			x	x	2

This is due to the morphological influence of each location. The Gunung Belumut waterfall is located upstream which the water level is higher due to raining season. The samplers were dislodged by the heavy flow stream and the analysis of sample could not be continued.

Table 2.3: Data tabulation of macroinvertebrates in Gunung Belumut Waterfalls

Upstream Gunung Belumut Waterfalls							
	Month	May	June	July	11-Aug	18-Aug	4-Sept
No.	Macroinvertebrates Name	Abundance					
1	Stonefly nymph	x	MCO	MCO	3	1	x
2	Whirligig beetle larvae	x			1	16	x
3	Prong-gilled mayfly	x			2	x	x
4	Common net-spinner	x			1	x	x
5	Two-tailled demoiselle	x			1	x	x
6	Long-headed caddisfly	x			1	2	x
7	Flattened mayfly	x			1	x	x
8	Spiny crawling mayfly	x			x	1	x
9	Non-bitting midge larvae	x			x	1	x
10	Common dragonfly	x			x	1	x
11	Cranefly larvae	x			x	1	x
12	Balloon-tailed damselfly	x			x	1	x
13	Shrimp	x			x	3	x
14	mystery snail	x			x	2	x
15	River prawn	x			x	1	x

While at Ulu Dengar River, this stream is located in the middle part of the stream flow. The size and structure of this river are smaller than waterfall. The substrate of the river consists of sand particles and muddy wooden logs. While at Gunung Belumut waterfall have a large boulder all over the stream flow. The habitual influence of macroinvertebrates preferences influences the colonization to the sampler submerged in the stream flow.

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