

Does the exclusion of meiofauna affect the estimation of biotic indices using stream invertebrates?

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Biomonitoring of rivers is usually undertaken using information based on macroinvertebrate assemblages. However, exclusion of meiofauna (i.e. invertebrates less than 0.5 mm in size) when sorting benthic invertebrates can affect the estimation of densities and other biotic indices. In the present study, the effect of excluding the less than 0.5 mm fraction of invertebrates on estimation of benthic invertebrate indices was investigated in the Naro Moru River, Kenya. The Shannon–Wiener diversity index, Pielou's evenness index, a multimetric index, Simpson's diversity index, Margalef's diversity index, mean invertebrate density, taxa richness, and Ephemeroptera, Plecoptera and Trichoptera (EPT) densities were determined. Only mean invertebrate and EPT densities differed significantly between the greater than 0.5 mm and total fractions. In conclusion, exclusion of meiofauna from invertebrate samples can affect the estimation of some stream invertebrate biotic indices.

Keywords: biomonitoring, Kenya, macroinvertebrate, river, sieve mesh size

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Introduction

Stream invertebrate communities change in response to change in physicochemical habitat factors, thus providing a useful tool for assessment of biotic structure and water quality in streams (Hussain and Pandit 2012; Herman and Nejadhashemi 2015). Invertebrates also perform important ecosystem processes, contributing to nutrient fluxes, and provide food to higher trophic levels (Covich et al. 1999). Benthic invertebrates of a size greater than 0.5 mm are the most commonly applied indicators of freshwater ecosystems' ecological condition (Allan and Castillo 2007). However, meioinvertebrates, of size less than 0.5 mm, are normally the most abundant and diverse in benthic samples, and contribute greatly to benthic ecosystem functioning (Palmer 1990; Vincete 2010). Thus, more information can be obtained from studies that consider meioinvertebrates than studies that are restricted to macroinvertebrates. This knowledge is required to assess whether additional effort is necessary, and the extent of sampling bias expected when not using methods that simplify invertebrate samples processing (Oliver and Beattie 1996).

Various techniques, such as sieving and subsampling, aimed at reducing the cost and processing time of invertebrate samples, have been developed in recent decades (Growth et al. 1997). However, the methods used during processing of invertebrate samples may affect the

estimation of invertebrate density and composition, and consequently the accuracy of other biotic indices (Crewe et al. 2001; Mbaka et al. 2014). For example, Mbaka et al. (2014) evaluated the effect of sieve mesh size on the estimation of stream invertebrate density and composition. The authors found that the density of invertebrates retained by the coarse mesh sieve, size 0.5 mm, was four-fold lower than that retained in the total sample. Hartwell and Fukuyuma (2015) assessed the effect of sieve mesh size (i.e. 0.5 and 1 mm) on benthic invertebrate community composition and found that utilisation of a 1 mm mesh sieve was more biased, compared with the 0.5 mm mesh sieve, in estimation of biotic indices. Another study assessed the effect of sieve mesh size (0.5, 1 and 2 mm) on description of invertebrates and found that taxa richness, density and a multimetric index were higher in the smaller (0.5 mm) mesh sieve (Pinna et al. 2013). Therefore, choice of sieve mesh size may significantly influence the estimation of invertebrate density, composition and other biotic indices. This may result in misinformation that does not correctly answer biomonitoring research questions.

Various indices are used to assess the distribution of invertebrates in relation to river habitat quality. Examples of indices include the Shannon–Wiener diversity index, taxa richness, Pielou's evenness index, multimetric indices, and

Ephemeroptera, Plecoptera and Trichoptera (EPT) richness (Herman and Nejadhashemi 2015). The diversity index assesses different types of a data group, and the characteristics of a population, such as number of existing species (richness), equality in distribution of individuals (evenness) and total number (abundance) of organisms (Wilhm and Dorris 1968). Multimetric indices combine several single invertebrate metrics to evaluate river ecological condition (Herman and Nejadhashemi 2015). Less disturbed habitats are typically characterised by high values of biotic indices such as multimetric indices, EPT richness and diversity (Masese et al. 2009; Edegbene et al. 2015).

Kenyan rivers provide important ecosystem services, such as the provision of water for local communities and habitat for a diverse range of organisms (Mathooko et al. 2009). However, there is still a paucity of information on human impacts in these systems and how best to detect changes. Accurate description of invertebrate assemblages in such lotic ecosystems may aid in the assessment of water and ecological conditions, and provide baseline information that will help improve river conservation. The objectives of the current study were to assess the effect of (1) stream habitat conditions on stream invertebrate communities structure (e.g. composition), and (2) exclusion of meiofauna on estimation of invertebrate biotic indices in study sites with varied human disturbances along the Naro Moru River, Kenya.

Methods

Study river and sites characterization

The study was carried out at the Naro Moru River, Kenya, between 9–13 June and 2–7 September 2011, to detect potential seasonal differences in invertebrates assemblage structure (Bêche et al. 2006). The Naro Moru River is a second-order (Strahler order; Strahler 1957) stream formed when the North and South Naro Moru Rivers meet at approximately 2 180 m above sea level (asl). Rain in the catchment normally falls between October and December, and between March and May. The highest amount of rainfall (~160 mm) in the study area typically falls between March and May, during which peak discharge is about 2.5 m s⁻¹ (Mathooko 1998). Three study sites – upstream, midstream and downstream – were selected for this study based on observable human impacts. The upstream study site (Naro Moru US) was located in an area with dense vegetation canopy cover at the slopes of Mount Kenya. The surrounding area was forested (Supplementary Figure S1) and human impacts were minimal. The midstream site (Naro Moru MS) was located near Naro Moru township, and was used as a source of water for domestic and livestock use. The most common vegetation type was *Syzigium cordatum* trees. At the downstream site (Naro Moru DS), livestock were frequently brought to drink water.

Habitat assessment and invertebrates sampling and processing

Dissolved oxygen concentration, electrical conductivity, temperature and pH were measured using portable sensors. River width and water depth were determined using a tape measure and a graduated rod, respectively. Vegetation canopy cover and coverage of benthic

substrates were determined visually (Jennings et al. 1999; Silva et al. 2014). Current velocity was determined at 60% of total hydraulic depth with a flow meter and water discharge was computed using the velocity, width and depth measurements (Gordon et al. 2004). Habitat measurements were made once at every site before sampling.

Stream benthic invertebrates were collected using a Hess sampler (effective working area: 0.029 m², 100 µm mesh size) in June and September 2011. Collections of invertebrates began from a downstream sampling location at each site. The Hess sampler was put at the bottom of the river at each sampling point with the open side facing upstream and substrates confined by the sampler were disturbed by hand for 3 min. Materials retained in the net of the sampler were carefully removed, placed in labelled plastic bags and preserved using formalin solution (4%). A total of 10 samples were collected at every site. In the laboratory, samples were washed with water through a 0.5 mm mesh sieve. This created an invertebrate fraction retained by the sieve (greater than 0.5 mm) and another one filtered through it (less than 0.5 mm) into a 100 µm mesh sieve. The sum of the two fractions is hereafter referred to as the 'total fraction'. The invertebrates in different fractions were identified under a dissecting microscope to order and family levels following Gerber and Gabriel (2002), counted and the density values expressed per unit area (individuals m⁻²). Several biotic indices were computed, including the Shannon–Wiener diversity index, Pielou's evenness index, Simpson's and Margalef's indices of diversity, and a multimetric index. The Shannon–Wiener diversity index (H') was calculated as follows:

$$H' = -\sum \left[\frac{n_i}{N} \times \ln \left(\frac{n_i}{N} \right) \right] \quad (1)$$

where n_i is the number of individuals belonging to species i and N is the total number of individuals (Shannon and Wiener 1949). Pielou's evenness index (J' ; Pielou 1966) was calculated as follows:

$$J' = H'/\ln(S) \quad (2)$$

where S is the total number of species. The Simpson's index of diversity (S' ; Simpson 1949) was calculated as given in the following equation:

$$S' = 1 - \sum n_i(n_i - 1)/N(N - 1) \quad (3)$$

where n is the number of individuals belonging to species i and N is the total number of individuals. Margalef's index of diversity (D) was calculated as follows:

$$D = S - 1/\ln(N) \quad (4)$$

where S is the total number of species and N is the total number of individuals.

A multimetric index was calculated as described by Ziglio et al. (2006). This multimetric index calculates the status of biotic communities based on the ecological quality ratio, i.e. the ratio between the observed conditions and a predicted reference condition (Søndergaard et al. 2005;

Table 2: Mean densities (individuals m⁻²) of benthic invertebrate taxa at the Naro Moru River upstream (US), midstream (MS) and downstream (DS) retained in the greater than 0.5 mm and less than 0.5 mm fractions, and percentage of invertebrates lost (% lost) when only considering the invertebrates retained in the greater than 0.5 mm fraction. The value in parentheses is the SD

Taxa	Naro Moru US			Naro Moru MS			Naro Moru DS		
	>0.5 mm	<0.5 mm	Lost (%)	>0.5 mm	<0.5 mm	Lost (%)	>0.5 mm	<0.5 mm	Lost (%)
Baetidae	3 413.7 (187)	1 724.1 (273)	33.5	2 275.8 (191)	1 758.6 (221)	43.6	1 413.7 (169)	2 241.3 (261)	61.3
Heptageniidae	34.5 (0)	0 (0)	0	68.9 (0)	0 (0)	0	172.4 (0)	0 (0)	0
Caenidae	34.5 (0)	0 (0)	0	172.4 (17)	0 (0)	0	103.4 (0)	0 (0)	0
Chironomidae	1 724.1 (164)	7 000 (772)	80.2	965.5 (69)	2 620.7 (113)	73.1	3 862.1 (392)	7 103.4 (521)	64.7
Ceratopogonidae	34.5 (0)	0 (0)	0	0 (0)	0 (0)	0	34.5 (0)	0 (0)	0
Simuliidae	0 (0)	0 (0)	0	34.5 (0)	0 (0)	0	0 (0)	0 (0)	0
Plecoptera	1 655.2 (226)	2 241.4 (252)	57.5	2 344.8 (186)	344.8 (48)	12.8	103.4 (24)	0 (0)	0
Zygotera	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0
Hydropsychidae	0 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	344.8 (0)	100
Elmidae	0 (0)	0 (0)	0	0 (0)	68.9 (0)	100	103.4 (0)	172.4 (0)	62.5
Helodidae	34.5 (0)	0 (0)	0	0 (0)	0 (0)	0	0 (0)	0 (0)	0
Ostracoda	0 (0)	275.9 (0)	100	0 (0)	137.9 (0)	100	0 (0)	0 (0)	0
Hemiptera	0 (0)	0 (0)	0	68.9 (0)	0 (0)	0	34.5 (0)	0 (0)	0
Sphaeriidae	103.4 (24)	0 (0)	0	0 (0)	0 (0)	0	68.9 (0)	0 (0)	0
Oligochaeta	0 (0)	0 (0)	0	34.5 (0)	1 517.2 (278)	97.7	862.1 (235)	7 068.9 (914)	89.1
Hydracarina	0 (0)	862.1 (352)	100	0 (0)	0 (0)	0	0 (0)	0 (0)	0
Potamoneutidae	0 (0)	0 (0)	0	0 (0)	0 (0)	0	34.5 (0)	0 (0)	0

Table 3: Mean values for biotic indices for invertebrates retained in the greater than 0.5 mm and total fractions in the Naro Moru River upstream (US), midstream (MS) and downstream (DS) study sites. The value in parentheses is the SD

Index	Naro Moru US				Naro Moru MS				Naro Moru DS			
	June		September		June		September		June		September	
	>0.5	Total	>0.5	Total	>0.5	Total	>0.5	Total	>0.5	Total	>0.5	Total
Shannon–Wiener diversity	0.6 (0.06)	0.9 (0.03)	0.6 (0.1)	0.7 (0.08)	0.6 (0.1)	0.9 (0.4)	0.5 (0.1)	0.6 (0.02)	0.5 (0.4)	0.7 (0.4)	0.6 (0.07)	0.5 (0.2)
Multimetric index	0.5 (0.1)	0.6 (0.1)	0.7 (0.1)	0.7 (0.05)	0.4 (0.07)	0.5 (0.1)	0.8 (0.05)	0.7 (0.04)	0.2 (0.1)	0.3 (0.1)	0.5 (0.2)	0.5 (0.2)
Pielou's evenness	0.6 (0.2)	0.8 (0.1)	0.6 (0.07)	0.5 (0.1)	0.6 (0.2)	0.7 (0.2)	0.5 (0.1)	0.5 (0.2)	0.4 (0.3)	0.5 (0.3)	0.4 (0.20)	0.4 (0.2)
EPT density	268.9 (136.6)	496.5 (422.0)	758.6 (387.1)	1 324.1 (323.1)	117.2 (57.7)	262.0 (244.5)	855.2 (301.9)	1 130.9 (217.3)	34.5 (34.5)	131.0 (147.1)	324.1 (245.7)	772.4 (636.1)
Total density	379.3 (172.4)	781.4 (631.1)	1 027.5 (474.9)	2 986.1 (1 091.1)	275.8 (114.3)	903.4 (608.2)	917.2 (292.2)	1 579.3 (421.9)	241.4 (235.1)	1 644.6 (2 025.3)	1 124.1 (516.4)	3 124.1 (1 779.2)
Taxa richness	2.8 (0.4)	3.2 (0.4)	3.2 (0.3)	4 (1)	3.6 (1.1)	4 (1.5)	3.2 (1.1)	4 (0.7)	2.4 (1.5)	3.4 (1.5)	4.4 (1.8)	4.8 (1.5)
Pooled richness	14	16	16	20	18	20	16	20	12	17	22	24
Simpson's diversity	0.5 (0.1)	0.5 (0.03)	0.6 (0.1)	0.6 (0.02)	0.6 (0.10)	0.4 (0.02)	0.6 (0.04)	0.7 (0.04)	0.4 (0.30)	0.4 (0.22)	0.5 (0.2)	0.5 (0.2)
Margalef's diversity	0.8 (0.2)	0.8 (0.2)	0.7 (0.2)	0.7 (0.2)	1.3 (0.4)	0.9 (0.3)	0.7 (0.3)	0.8 (0.2)	0.8 (0.6)	0.7 (0.5)	1.0 (0.6)	0.9 (0.4)

Mean invertebrate densities in the total fraction ranged from 781.4 ± 631.1 individuals m⁻² at Naro Moru US in June to 3 124.1 ± 1 779.2 individuals m⁻² at Naro Moru DS in September (Table 3), and differed significantly between the greater than 0.5 mm and total fractions and seasons (all $p < 0.05$ in GLMM; all $p < 0.001$ in pairwise comparisons with Tukey contrasts; Table 4). The density of EPT taxa differed significantly between the greater than 0.5 mm and total fractions ($p = 0.004$ in LMM) and the Shannon–Wiener diversity index was close to significance ($p = 0.06$). However, no statistically significant difference was observed for the multimetric index, Pielou's evenness index, taxa richness, and Margalef's and Simpson's diversity indices (all $p > 0.05$

in LMM). Tukey contrasts showed that the total fraction had higher mean values for EPT taxa densities ($p < 0.05$). Season had a statistically significant influence on most biotic indices (all $p < 0.05$ in LMM), but not on the Shannon–Wiener ($p = 0.14$) and Margalef's ($p = 0.29$) diversity indices. The season × sieve mesh size interaction term was insignificant (all $p > 0.05$; see Table 4 for GLMM results).

Habitat conditions

The altitude (m asl) of the study sites ranged from 1 941 m (Naro Moru DS) to 2 223 m (Naro Moru US). The Naro Moru US study site had the highest canopy cover intensity (90%), whereas Naro Moru MS had the lowest (1%) canopy

Table 4: *F* and *p*-values for the mixed effects models testing the effects of sieve mesh size and season on invertebrate communities biotic indices

Index	Sieve mesh size		Season		Sieve mesh size × season	
	<i>F</i> _{1,56}	<i>p</i>	<i>F</i> _{1,56}	<i>p</i>	<i>F</i> _{1,56}	<i>p</i>
Shannon–Wiener diversity	7.1	0.06	3.3	0.14	3.1	0.56
Multimetric index	5.1	0.08	59.0	0.0008	4.0	0.4
Pielou's evenness	0.9	0.96	6.2	0.03	3.2	0.6
EPT density	13.9	0.004	67.2	0.001	3.0	0.56
Total density	9.5	0.01	12.3	0.006	1.2	0.92
Taxa richness	6.2	0.07	7.6	0.03	0.01	1
Simpson's diversity	0.2	0.96	10.1	0.01	0.8	1
Margalef's diversity	0.8	0.96	1.2	0.29	0.7	1

Table 5: Coordinates and water physicochemical variables measured at the Naro Moru River upstream (US), midstream (MS) and downstream (DS) study sites. na = Not available

	Naro Moru US		Naro Moru MS		Naro Moru DS	
Coordinates	00°10'45.2" S, 37°06'43.6" E		00°09'32.8" S, 37°01'14.8" E		00°08'46.9" S, 37°00'32.0" E	
Altitude (m asl)	2 223		1 980		1 941	
Canopy cover (%)	90		1		5	
Study site dimensions (m)	Width 6.5, length 15, depth 0.4		Width 5, length 25, depth 0.3		Width 5, length 20, depth 0.2	
Major substratum (%)	Boulders and cobbles; 90		Boulders and cobbles; 80		Cobbles; 90	
	June	September	June	September	June	September
Temperature (°C)	13	13.5	19.4	20	20.1	17.5
Dissolved oxygen (mg L ⁻¹)	7.4	8.2	7.3	7.9	7.1	8
Conductivity (μS cm ⁻¹)	86.1	31.6	86.9	56.6	84.9	62.8
pH	7.4	8	7.3	7.9	7	8
Velocity (m s ⁻¹)	0.1	na	0.4	na	0.6	na
Discharge (m ³ s ⁻¹)	0.4	na	0.9	na	1	na

cover intensity (Table 5). The Naro Moru MS and DS study sites had the same river width (5 m), whereas the Naro Moru US river site was 6.5 m wide. Water depth varied from 0.2 m (Naro Moru DS) to 0.4 m (Naro Moru US). Generally, the most common (>80%) substrate type in the study sites was cobbles and boulders. Water temperature ranged from 13.0 °C (Naro Moru US) to 20.1 °C (Naro Moru DS). Dissolved oxygen concentrations varied from 7.1 mg L⁻¹ (Naro Moru DS) to 8.2 mg L⁻¹ (Naro Moru US), whereas pH, specific conductivity, water velocity and discharge ranged from 7.0 (Naro Moru DS) to 8.0 (Naro Moru US/DS), 31.6 μS cm⁻¹ (Naro Moru US) to 86.9 μS cm⁻¹ (Naro Moru MS), 0.1 m s⁻¹ (Naro Moru US) to 0.6 m s⁻¹ (Naro Moru DS) and 0.4 m³ s⁻¹ (Naro Moru US) to 1.0 m³ s⁻¹ (Naro Moru DS) (Table 5).

Discussion

Invertebrates assemblage and biotic indices

Exclusion of small-sized invertebrates during processing of samples can affect estimation of invertebrates composition and biotic indices. In the current study, retention of Ostracoda and Hydracarina in the less than 0.5 mm fraction can be attributed to their small body size, which enabled them to pass through the 0.5 mm mesh sieve. Oligochaetes and chironomids were also primarily (>80%) retained in the less than 0.5 mm fraction. The small body size of chironomids, and absence of appendages on oligochaetes body, may have better enabled them to pass

through the 0.5 mm mesh sieve than larger invertebrates (e.g. Heptageniidae) (Tanaka and Leite 1998). Exclusive retention of hydropsychids in the less than 0.5 mm fraction can be attributed to factors such as seasonal dominance of small-sized individuals or retardation in growth (Mesa 2012).

Sieve mesh size had a statistically significant influence on estimation of mean densities of invertebrates (e.g. EPT). Specifically, upstream and downstream sites had high densities of some major invertebrate taxa retained in the less than 0.5 mm fraction (Figure 1). However, sieve mesh size had no significant effect on estimation of indices such as Simpson's index of diversity and a multimetric index. This suggests that, although exclusion of the less than 0.5 mm fraction may have a significant effect on estimation of mean invertebrate density and composition (Mbaka et al. 2014), the effect on indices such as Simpson's and Margalef's diversity indices may be insignificant (e.g. Tanaka and Leite 1998), perhaps because taxa richness also did not differ significantly. Therefore, accurate description of invertebrate density and composition require consideration of small-sized invertebrates (Crewe et al. 2001). In particular, identification of invertebrates in the field during rapid bioassessment may be greatly affected if certain species are lost in great numbers, when using a sampler with a bigger net size (e.g. 1 mm). Exclusion of small-sized invertebrates may also result in misinformation if sampling coincides with a period of the year with high densities of small-sized invertebrates, as observed in this study, and in study sites dominated by small species. However, exclusion of small-sized

invertebrates from invertebrate samples may not affect the accuracy of biomonitoring information when considering some diversity indices, or in development and application of multimetric indices (Masese et al. 2009; Aarnio et al. 2011). Moreover, choice of sieve mesh size may depend on the type of invertebrates targeted for sampling, and life stage (e.g. adults), given that some invertebrate species may be larger than 0.5 mm (Aarnio et al. 2011). Indeed, processing the invertebrates retained in small-sized mesh sieves (e.g. <0.5 mm) has some disadvantages, such as the longer time required to process samples and the challenge of identifying samples to lower taxonomic levels (Barba et al. 2010). Moreover, invertebrate larvae may have less synchronised emergence times and future studies should consider taking samples the entire year to avoid biased results (Dudgeon 2000). In addition, future studies should consider identifying invertebrates to higher levels of taxonomic resolution because different species may have varied niche requirements, which may not be evident at coarser levels of identification (e.g. family), and are a better estimate of true ecosystem biodiversity (Prance 1994; Lenat and Resh 2001).

The high mean density of invertebrate taxa, such as oligochaetes, at the downstream site may be due to their ability to withstand the disturbed environment created by domestic animals that come to drink water. Domestic animals may increase conductivity and sedimentation, and river substrates compaction, through trampling and introduction of faeces in the riparian and in-stream areas (e.g. Scrimgeour and Kendall 2003; Niyogi et al. 2007), thereby negatively affecting the density of sensitive taxa such as EPT (Table 3; Braccia and Voshell 2007; Burdon et al. 2013). Consequently, invertebrate communities in disturbed sites may be more similar due to homogenisation by human-induced pressures (Feio et al. 2015).

The EPT taxa are widely applied to assess human activities in aquatic ecosystems and are a reliable index that is sensitive to change in habitat quality (Edegbene et al. 2015). On the other hand, taxa such as chironomids are able to withstand varied habitat conditions (Selvanayagam and Abril 2015). For example, Edegbene et al. (2015) investigated the effect of human activities on a river using macroinvertebrates and found that the relative abundances of EPT taxa were significantly reduced in the most impacted sites. Aazami et al. (2015) also found that the EPT taxa, and a multimetric index, identified study sites that were impacted by human activities, such as sand mining.

Habitat conditions

The high mean water temperature recorded at the Naro Moru MS and DS sites, compared with the Naro Moru US site, is most likely caused by the low (1%) riparian vegetation canopy cover recorded at this site. For example, Bowler et al. (2012) performed a meta-analysis on the effect of riparian vegetation canopy cover on stream water temperature and found that stream areas with low riparian vegetation canopy cover had higher mean water temperature. The high conductivity at mid- and downstream sites may be due to increased intensity of human-related perturbations (Herbst et al. 2012; Burdon et al. 2013). Riparian vegetation canopy cover also influences chemical water quality

(Mayer et al. 2007). For example, de Souza et al. (2013) investigated the effect of riparian vegetation on water quality and found that water chemistry (e.g. conductivity and dissolved oxygen) was significantly influenced by riparian vegetation characteristics, such as canopy cover. Increase in discharge at the mid- and downstream sites may be as a result of water input from ground water sources and run off from anthropogenic sources.

Conclusions

In conclusion, exclusion of meiofauna (<0.5 mm) had a statistically significant influence on estimation of invertebrate composition and density. However, some indices (e.g. Simpson's diversity index) were not significantly influenced. It is recommended that meiofauna should be taken into account if the objective of the study is to monitor river conditions based on invertebrate density and composition. Conservation measures should be put into place to protect river sites from human-induced water and habitat quality changes, and invertebrate biodiversity change.

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