Effect of methodology and sampling time on the taxa richness of aquatic macroinvertebrates and subsequent changes in the water quality index from three tropical rivers, Costa Rica

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Received 02-III-2005. Corrected 05-V-2008. Accepted 03-VI-2008.

Abstract: Aquatic macroinvertebrates have proven to be a useful tool for water quality studies and biomonitoring in temperate areas. Recently, efforts have been made to adapt these methods to tropical environments, but there are still uncertainties concerning the most adequate methodology and collecting time. Three rivers, one in Alajuela province and two in Puntarenas province in Costa Rica, were tested with two methods and different collecting times, in the rainy and dry season. The first method involved collecting of organic and inorganic material for a fixed time period (3, 5, 8, 10 min) with a strainer. This material was transferred to a plastic bowl containing 70% alcohol and aquatic macroinvertebrates were sorted out in the laboratory. With the second method the specimens were collected in the field directly out of the strainer for a total collecting time of 120 minutes and preserved immediately with 70% alcohol. In order to obtain species accumulation curves for this method, subsamples were taken every 15 minutes. The data analysis showed that the abundance and taxa richness was higher with the second method, and a higher number of genera could be found with increasing collecting time, but not necessarily a higher number of individuals. A difference in the number of individuals between rainy and dry season was observed. Species accumulation curves for samples taken with both methods showed that new genera and families were still being found after the maximum time of collection, no matter which season or river. Categories of water quality obtained from the BMWP-CR index varied greatly among sampling times and methods used. The second method always achieved a higher water quality than the longest sampling time (10 min) in the first method. However, it still didn't reach the level obtained for all families found in both methods combined. Although the first method is the one officially used in most sampling protocols for biomonitoring in temperate zones, these results suggest that more extensive testing of adequate sampling time and methodology is still necessary for tropical rivers. Rev. Biol. Trop. 56 (Suppl. 4): 257-271. Epub 2009 June 30.

Key words: biomonitoring, aquatic macroinvertebrates, water quality, species accumulation curve, river ecology, Costa Rica.

Many studies have been performed around the world using aquatic macroinvertebrates as bioindicators in order to monitor the water quality of surface water. This group of organisms is especially useful for biomonitoring due to their relatively sedentary behavior, long life spans and nearly similar abundance around the world (Schwoerbel 1999, Rosenberg and Resh 1993). In addition, collection and identification techniques are inexpensive in comparison to chemical analysis and other methods of water testing. Also, biomonitoring with macroinvertebrates shows long term changes, while chemical analysis can be considered a "snapshot" and reflects only the momentary water quality situation (Alba-Tercedor 1996). All together, these characteristics make benthic macroinvertebrates especially advantageous for water quality evaluation. Since aquatic insects evolved over a similar time period all over the world, some authors consider that methods using these organisms as bioindicators, are also compatible around the world (Schwoerbel 1999).

The use of aquatic insects as bioindicators had been studied primarily in temperate zones such as Europe and the United States, and there is still a lack of knowledge of benthic macroinvertebrate ecology and distribution in the neotropics (Roldán 1992) which makes such evaluation of the water difficult, but necessary. Due to this necessity, the study of aquatic insects has escalated in Costa Rica in the past ten years, but there is still relatively little literature available. A first step that helps avoiding misidentification of aquatic insects lies in the establishment of a reference collection, and the publication of a list of the taxa encountered in the different aquatic systems (Springer 1998). This represents an invaluable resource for biomonitoring studies, among others.

There are only a few publications on biomonitoring in Costa Rica and other Central American countries (eg. Charpentier and Tabash 1988, Michels 1998, Paaby *et al.* 1998, Fenoglio *et al.* 2002, Mafla-Herrera 2005), and the necessity for calibration of methodologies used in temperate rivers and streams to tropical conditions has been realized and documented (Fenoglio *et al.* 2002, Stein *et al.* 2008). Because of its importance, limnologists and biologists in Costa Rica have focused more and more on biomonitoring over the past five years, but no standard method has yet been developed. Therefore, this investigation seeks to compare two different methodologies and the effect of sampling time in order to find the most efficient and useful method and the most effective time of collecting. One aspect of the methodology that is still unknown, is how much time is required to obtain adequate representation of the local insect biota, and if there are any differences in the effectiveness of collection time and methodology used between the rainy season and dry season. Collecting methodology consequently influences the results of the water quality index used and may result in different categories of water quality.

MATERIAL AND METHODS

Study areas: Aquatic macroinvertebrates were collected in three rivers in Costa Rica: Río Uvita, Río Balso and Río San Lorencito. Río Uvita lies in the province Puntarenas in the south-western lowlands of Costa Rica. The samples for this study were taken 2 km north-east of the town Uvita and about 4.5 km before the Río Uvita ends in the Pacific (09º 09´ N, 83º 44´ W; 10 m. above sea level). The area surrounding the study site is pasture land on one side, and farmland with shrub vegetation and some eucalyptus trees on the other. There is one small settlement (San Josecito) 6 km upstream from the investigated area. In the past, poison was used by locals to catch fish and shrimp in the river. The climate is classified as aseasonal humid with annual precipitations between 2500 to 3500 mm and an average annual temperature of 26 °C. The wet season is from May until December (700mm/month), and the dry season between January and April (50mm/month). Samples were taken for the rainy season on the 6th of Oct. 2003, and during the dry season on the 4th of Feb. 2004. The average width of Río Uvita shrank from 10 m to 3 m, and the average depth from 90 cm to 40 cm. The river-bed was mainly covered by stones and grit, and there was hardly any dead wood; water temperature was 27ºC in the rainy and 30ºC in the dry season.

The Río Balso lies in the same region as the Río Uvita, and samples were taken 12 km north of the settlement of Ojochal and about 20 km before the mouth of the river into the Pacific (09º 03´ N, 83º 09´ W; 180 m.a.s.l.). The study site is surrounded by primary rainforest, without any anthropogenic influence at this point or in the upstream area. Climate data are the same as for the Río Uvita. The samples were taken on the 30th of Nov. 2003 and on the 18th of Feb. 2004. The average width of the Río Balso changed from 2 m to 50 cm, and the depth from 20 cm to 10 cm. The substrate consisted of stones, grit, and organic matter in the form of dead wood and leafpacks in decomposition; the water temperature was 21ºC for both sampling dates.

The Río San Lorencito is located in Alajuela province in the northern highlands of Costa Rica. The climate is classified as aseasonal hyper humid with annual precipitation over 4000 mm and an average annual temperature of 21°C. In general the wet season is from June until September (800 mm/month), and the dry season from February to May (150 mm/month). The Río San Lorencito is a branch of the Río San Lorenzo and lies in the Cordillera de Tilarán. The samples were taken 100m upstream from the research station at the southeast border of the Biological Reserve Manuel Brenes (10°13' N, 84°37' W; 960 m.a.s.l.). The stream is surrounded by primary forest, and there is no antropogenic influence at or above the sampling site. Samples were taken on November 11th, 2003 and on March 13th, 2004. The average width (5 m) and depth (60 cm) were the same for both seasons, and precipitation was unusually high for this dry season. The substrate consisted of boulders, stones, gravel, sand and organic matter in the form of leafpacks in decomposition; a large amount of dead wood was also present. The current conditions varied among microhabitats, from fast-flowing riffles to calm pools; water temperature was 18ºC for both sampling dates.

Methods: For collection a mesh strainer with a mesh opening of 1mm, an inner diameter of 21cm and a depth of 10 cm was used. All microhabitats present were sampled on both sides of a river; the direction of collection was always upstream.

With the first method material from the different microhabitats of a river was collected during a predetermined collection time, and included organic and inorganic material, such as leaves, grit and sticks. The collection times were 3 min, 5 min, 8 min and 10 min, which means that four independent collections were obtained with the first method at each river. The collected material was transferred into a plastic bowl and 70% alcohol was added to preserve the material, including the insects. In the laboratory the material was searched for benthic macroinvertebrates which were placed in vials containing 70% alcohol for later identification.

The second method combines collecting and sorting of aquatic macroinvertebrates in the field. The total collection and sample picking time was 120 min at each river. During this time span, the material was collected with the net and the specimens sorted out with forceps and placed in glass vials with 70% alcohol for later identification in the laboratory. Each 15 min a new vial was used, dividing the total collection time of 120 min into eight sequences, (15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 105 min and 120 min). This method is the common method used for field projects by students and researchers in the Biology School at the University of Costa Rica.

Identification: The collected specimens were sorted and identified to the genus level (with exception of Chironomidae, Diptera) with the aide of a dissecting microscope, specialized literature, and identification keys (Roldán 1996; Merritt and Cummins 1996; Springer and Hanson in prep.). All specimens are deposited in the aquatic entomology collection, at the Museo de Zoología, Universidad de Costa Rica.

BMWP-CR index[1]: The BMWP' index (Biological Monitoring Working Party) was developed by Alba-Tercedor and Sánchez-Ortega (1988), based on the original index first described by Hellawell (1978). Recently efforts have been undertaken to adapt this index to the Costa Rican fauna (BMWP-CR index) in order to include it in the new Costa Rican water law, as an official measurement for biomonitoring.

1. During the process of publication the methodology for aquatic biomonitoring using the BMWP-CR index was published in the regulation Nr. 33903-MINAE-S (La Gaceta No. 178, 17. Sept. 2007)

TABLE 1
Categories of water quality defined by the BMWP values according to Alba-Tercedor (1996)

Water quality	BMWP	associated color
waters with excellent quality	> 120	blue
waters with good quality, no contaminations or obvious distortions	101 - 120	blue
waters with regular quality, eutrophic, medium contamination	61 - 100	green
waters with bad quality, contaminated	36 - 60	yellow
waters with bad quality, very contaminated	16 - 35	orange
waters with very bad quality, extremely contaminated	< 15	red

The levels of water quality are defined by the BMWP index as in Table 1. These levels are obtained by adding the sensitivity value (from 1-10) for each family found, independently of the abundance and generic diversity found in each family.

RESULTS

Abundance and taxa richness: For all sampling sites, a total of 89 genera were found representing 40 insect families, nine insect orders and five non insect classes (Table 2). The most diverse group was Coleoptera with 18 genera most of which belong to the family of Elmidae. A total of 3 214 individuals were collected in the three rivers, and the most abundant order was Ephemeroptera, which made up 42.4 % of the total number of individuals.

The abundance didn't necessarily increase with an increasing sampling time in the first method (Figs. 1A, C). In some cases (Río Uvita, Río San Lorencito) more than twice the number of individuals was found in 3 minutes than in 5 or 10 minutes. With one exception (Río Balso, rainy season), a

TABLE 2
Aquatic macroinvertebrates collected in Río Uvita, Río Balso and Río San Lorencito in both seasons with both methods

Taxa	Río Uvita	Río Balso	Río San Lorencito
Ephemeroptera			
Baetidae: *Americabaetis* sp.		X	
Baetidae: *Baetodes* sp.	X	X	X
Baetidae: *Camelobaetidius* sp.	X	X	X
Baetidae: *Cloeodes* sp.		X	
Baetidae: Gen. sp.		X	
Baetidae: *Mayobaetis* sp.		X	
Baetidae: *Moribaetis* sp.	X	X	X
Euthyplociidae: *Euthyplocia* sp.		X	
Leptohyphidae: *Haplohyphes* sp.	X	X	
Leptohyphidae: *Leptohyphes* sp.	X	X	X
Leptohyphidae: *Tricorythodes* sp.	X	X	X
Leptohyphidae: *Vacuperinus* sp.	X		
Leptophlebiidae: *Farrodes* sp.	X	X	X

TABLE 2 (Continued)

Aquatic macroinvertebrates collected in Río Uvita, Río Balso and Río San Lorencito in both seasons with both methods

Taxa	Río Uvita	Río Balso	Río San Lorencito
Leptophlebiidae: *Hagenulopsis* sp.	X		
Leptophlebiidae: *Thraulodes* sp.	X	X	X
Leptophlebiidae: *Traverella* sp.	X		
Plecoptera			
Perlidae: *Anacroneuria* sp.	X	X	X
Odonata			
Calopterygidae: *Hetaerina* sp.	X	X	X
Coenagrionidae: *Argia* sp.	X	X	X
Gomphidae: Gen. sp.		X	
Libellulidae: *Brechmorhoga* sp.		X	X
Libellulidae: *Perithemis* sp.			X
Megapodagrionidae: Gen. sp.	X	X	
Megapodagrionidae: *Heteragrion* sp.		X	
Megapodagrionidae: *Phylogenia* sp.		X	
Platysticitidae: *Perigomphus* sp.	X		
Polythoridae: *Cora* sp.	X	X	X
Hemiptera			
Hebridae: *Hebrus* sp.		X	
Naucoridae: *Ambrysus* sp.		X	
Naucoridae: *Limnocoris* sp.			X
Veliidae: *Rhagovelia* sp.	X	X	X
Megaloptera			
Corydalidae: *Corydalus* sp.	X	X	X
Corydalidae: *Chloronia* sp.			X
Trichoptera			
Calamoceratidae: *Phylloicus* sp.		X	X
Glossosomatidae: *Culoptila* sp.		X	X
Glossosomatidae: *Mexitrichia* sp.		X	X
Glossosomatidae: *Protoptila* sp.	X	X	
Hydrobiosidae: *Atopsyche* sp.			X
Hydropsychidae: *Calosopsyche* sp.		X	
Hydropsychidae: *Leptonema* sp.	X	X	X
Hydropsychidae: *Macronema* sp.		X	
Hydropsychidae: *Smicridea* sp.	X	X	X
Hydroptilidae: *Ochrotrichia* sp.			X
Leptoceridae: *Nectopsyche* sp.	X	X	X
Leptoceridae: *Oecetis* sp.	X		X
Philopotamidae: *Chimarra* sp.	X	X	X

TABLE 2 (Continued)

Aquatic macroinvertebrates collected in Río Uvita, Río Balso and Río San Lorencito in both seasons with both methods

Taxa	Río Uvita	Río Balso	Río San Lorencito
Polycentropodidae: *Polycentropus* sp.			X
Xiphocentronidae: Gen. sp.	X		
Lepidoptera			
Pyralidae: *Petrophila* sp.	X	X	
Family non det.			X
Coleoptera			
Dryopidae: *Dryops* sp.	X		
Elmidae: *Austrolimnius* sp.			X
Elmidae: *Cylloepus* sp.		X	X
Elmidae: *Disersus* sp.	X	X	X
Elmidae: *Heterelmis* sp.	X	X	X
Elmidae: *Hexacylloepus* sp.	X	X	
Elmidae: *Hexanchorus* sp.	X	X	X
Elmidae: *Macrelmis* sp.	X	X	X
Elmidae: *Neoelmis* sp.		X	
Elmidae: *Phanocerus* sp.	X	X	X
Elmidae: *Pharceonus* sp.			X
Elmidae: *Xenelmis* sp.			X
Curculionidae: Gen. sp.		X	
Lutrochidae: *Lutrochus* sp.	X	X	
Psephenidae: *Psephenops* sp.	X	X	X
Psephenidae: *Psephenus* sp.	X	X	X
Ptilodactylidae: *Anchytarsus* sp.		X	X
Staphylinidae: Gen. sp.			X
Diptera			
Athericidae: *Atherix* sp.			X
Ceratopogonidae: *Atrichopogon*? sp.	X		
Chironomidae	X	X	X
Empididae: *Hemerodromia* sp.		X	X
Empididae: *Neoplasta* sp.	X	X	X
Psychodidae: *Maruina* sp.			X
Simuliidae: *Simulium* sp.	X	X	X
Stratiomyidae: Gen. sp.		X	
Tipulidae Limoninae Gen. sp.			X
Tipulidae: Gen. sp.		X	X
Tipulidae: *Hexatoma* sp.	X	X	X
Tipulidae: *Limonia* sp.		X	
Tipulidae: *Molophilus* sp.			X
Tipulidae: *Tipula* sp.			X

TABLE 2 (Continued)

Aquatic macroinvertebrates collected in Río Uvita, Río Balso and Río San Lorencito in both seasons with both methods

Taxa	Río Uvita	Río Balso	Río San Lorencito
Other non insect groups			
Collembola		X	
Crustacea (Astacidea)	X	X	
Crustacea (Brachyura)		X	
Hidracarina		X	
Molusca: Gastropoda	X		
Oligochaeta	X	X	X
Tricladida: Planariidae	X	X	X

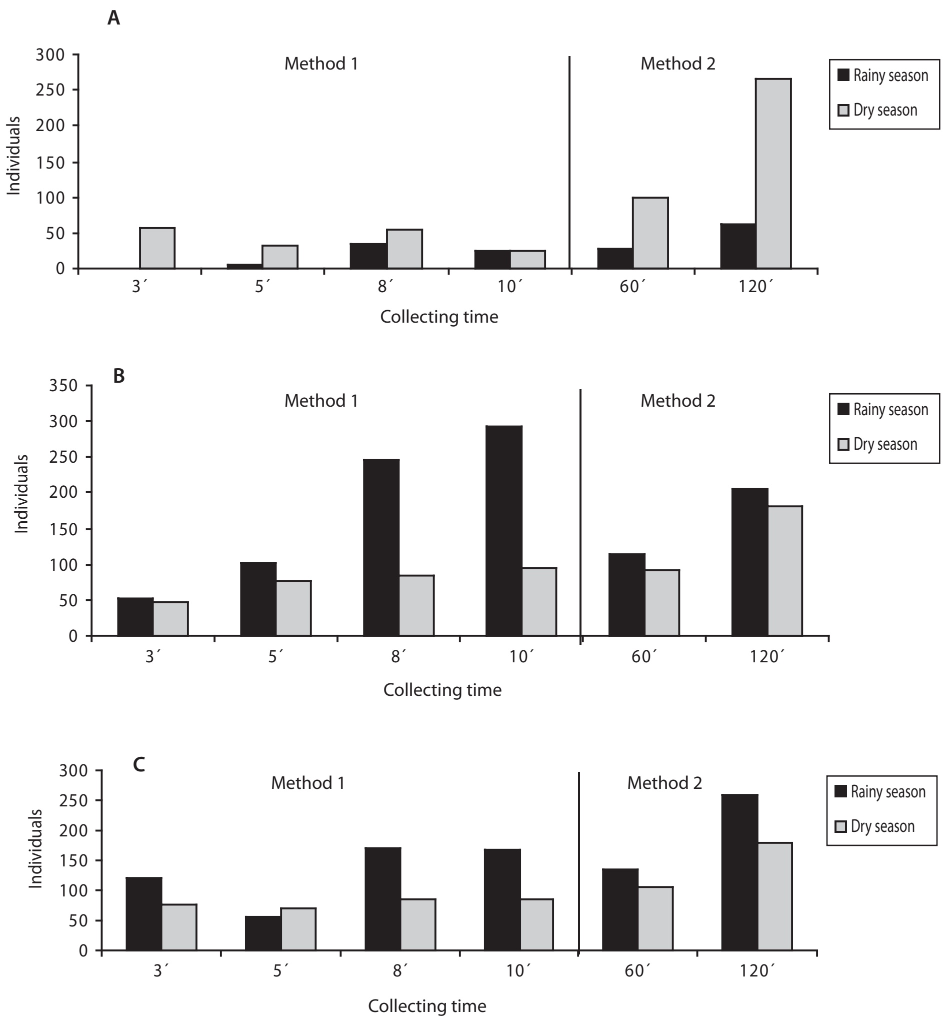

Fig. 1. Total number of individuals of aquatic macroinvertebrates collected in three rivers with both methods in both season: (a) Río Uvita, (b) Río Balso, (c) Río San Lorencito.

higher abundance was found with the second method. Even though the number of individuals didn't generally rise with an increasing sampling time (first method), there was an obvious tendency for a slight increase in the number of genera found with the collecting time (Figs. 2A, C). As for abundance, higher taxa richness was generally found with the second method. Comparing the number of genera found per season, a higher number was found in the Río Uvita in the dry season (Fig. 2A), but more genera were collected in the rainy season in the Río Balso and the Río San Lorencito (Fig. 2B, C).

Species accumulation curve: The accumulative number of genera and the accumulative number of families was usually higher in the dry season than in the rainy season no matter what method was used (Figs. 3-6). Exceptions are the Río Balso, where more genera and families were found with the first method in the rainy season (Fig. 3B), and the Río San Lorencito where one additional genus was found in the rainy season (Fig. 4C) than in the dry season with the second method (Fig. 6C). Species accumulation curves for samples taken with the first method show that new genera and families were still found after 10

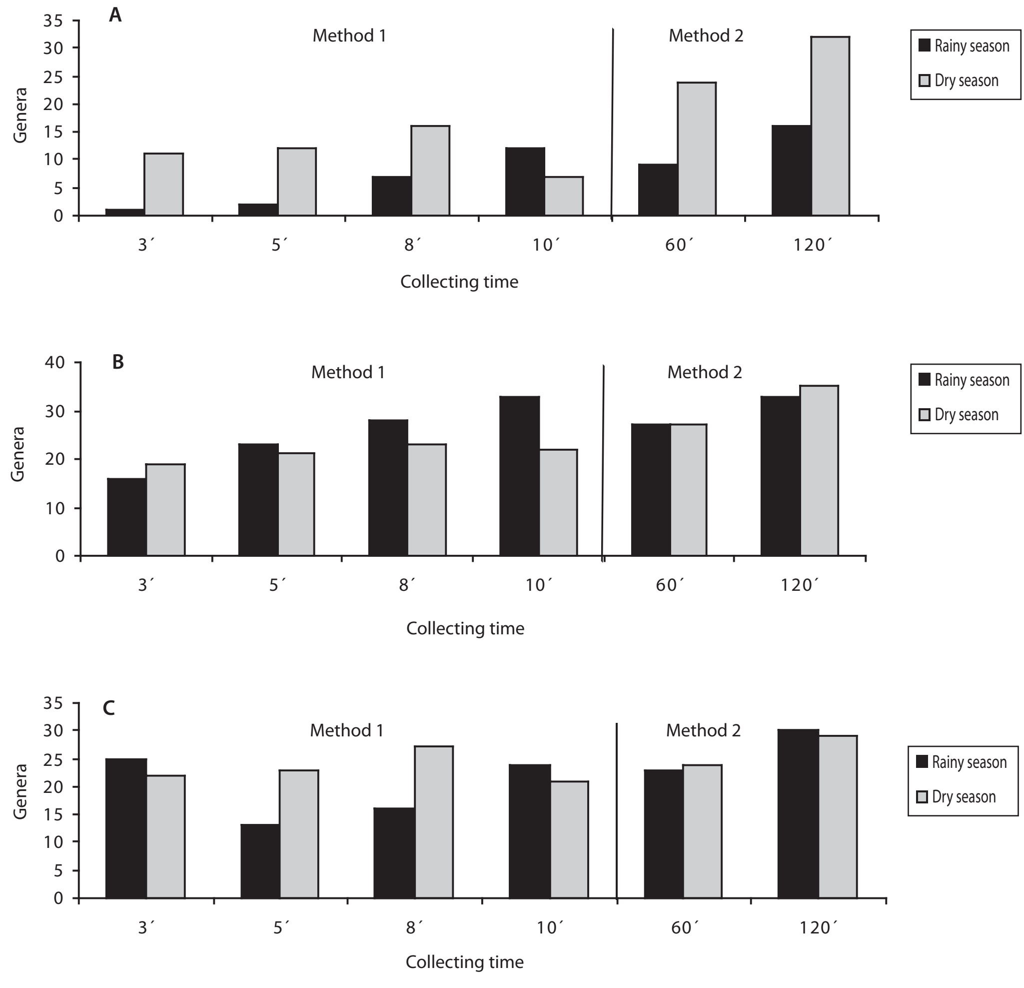

Fig. 2. Total number of genera of aquatic macroinvertebrates collected in three rivers with both methods in both seasons: (a) Río Uvita, (b) Río Balso, (c) Río San Lorencito.

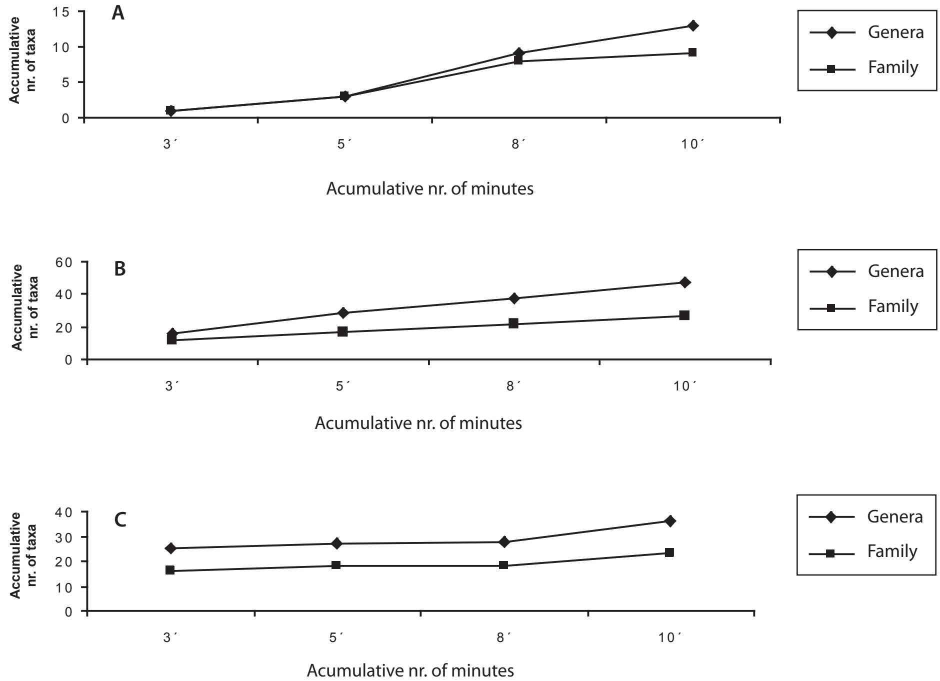

Fig. 3. Accumulative number of genera and families collected with the first method in the rainy season in the three rivers: (a) Río Uvita, (b) Río Balso, (c) Río San Lorencito.

minutes of collection (Figs. 3A, C and Figs. 5A, C) independent of season or river. Species accumulation curves for samples taken during 120 minutes with the second method show that new genera and families were still found after 120 minutes (Figs. 4A, C and Figs. 6A, C). One exception is the Río San Lorencito in the dry season. After 90 minutes no new genera and families were found (Fig. 6C).

BMWP-CR index: In general, categories of water quality obtained from the BMWP-CR index varied greatly among sampling times and methods used. With the second method the BMWP-CR index of all three rivers was always higher than with the first method (Figs. 7A, B). The greatest difference was in the Río Uvita in the dry season, which stayed in the yellow level (contaminated, bad water quality) with the first method, but reached the blue level (good water quality with no obvious contamination) with the second method (Fig. 7B). In the rainy season the situation was similar, although not as evident (Fig. 7A). The BMWP-CR index representing all families found in both methods combined was, for all three rivers, considerably higher than for each method alone (Figs. 7A, B).

A correlation between the number of genera and the BMWP-CR index was found (Pearson < 0.05). This is due to the fact that with a rising number of genera, more families are represented and therefore the BMWP-CR index is higher. None of the other parameters (individuals, rivers, seasons, and sampling time) showed any correlation (Pearson > 0.05) to the BMWP-CR index.

DISCUSSION

The fact that the number of collected aquatic macroinvertebrates is not correlated with the

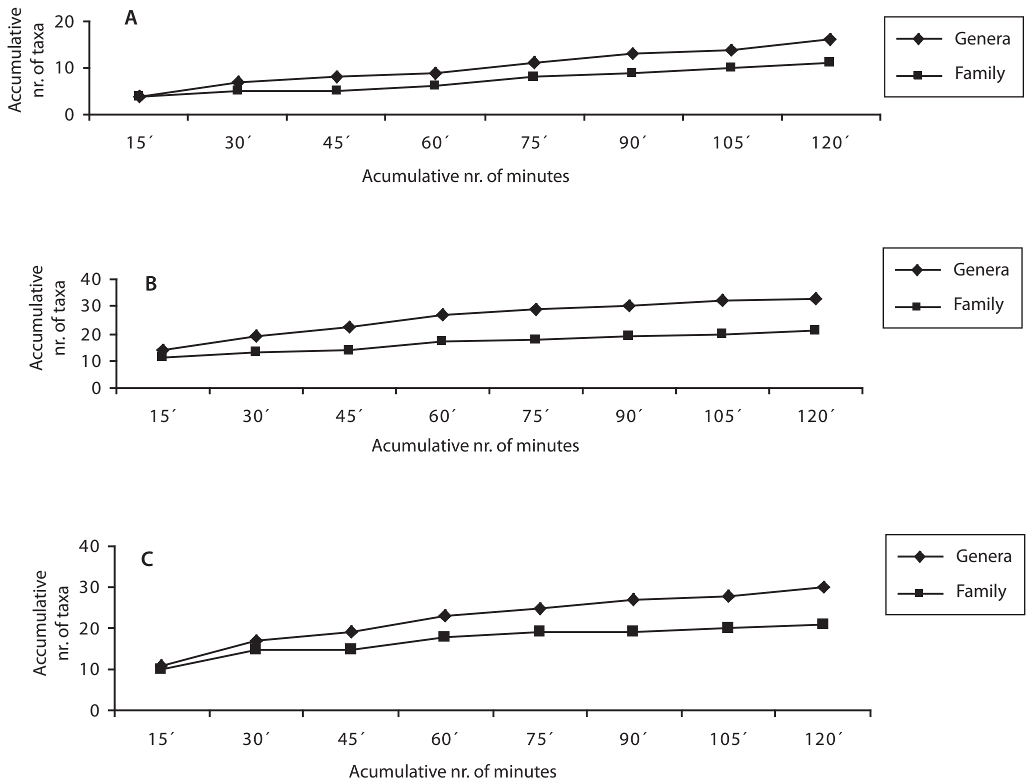

Fig. 4. Accumulative number of genera and families collected with the second method in the rainy season in the three rivers: (a) Río Uvita, (b) Río Balso, (c) Río San Lorencito.

sampling time in the first method, and in some cases even more individuals were found with a shorter collecting time, may be explained by the following. 1) When a "hot spot" of diversity like a leaf packet was collected in a short time sample, the number of individuals was relatively high, while this is evened out in the 8 or 10 min sample, where poor areas such as sandbanks were also included. 2) Flying insects such as adult Coleoptera (especially Elmidae) could have escaped from the net while collecting for a longer time, and small aquatic macroinvertebrates, such as Trichoptera and Diptera larvae (especially the very small stages), could crawl through the meshes. 3) When collecting for 8 or 10 min the net became packed with inorganic and organic material, so that it became difficult to keep the following material in the net. 4) Voracious predators such as Megaloptera or Odonata larvae were observed feeding on other macroinvertebrates after being collected, even when 70 % alcohol was added to the collecting bowl. 5) Perhaps the greatest problem was the transportation from the field to the laboratory, since the inorganic material such as stones and sand, may have ground the fragile macroinvertebrates. This damage often makes identification difficult.

The slight increase in the number of genera found with the amount of time collected, could be due to the greater amount of microhabitats sampled, since a greater area was covered with a higher sampling time. That would also explain why more genera were generally found with the second method, because here a much longer distance along the river was covered during the

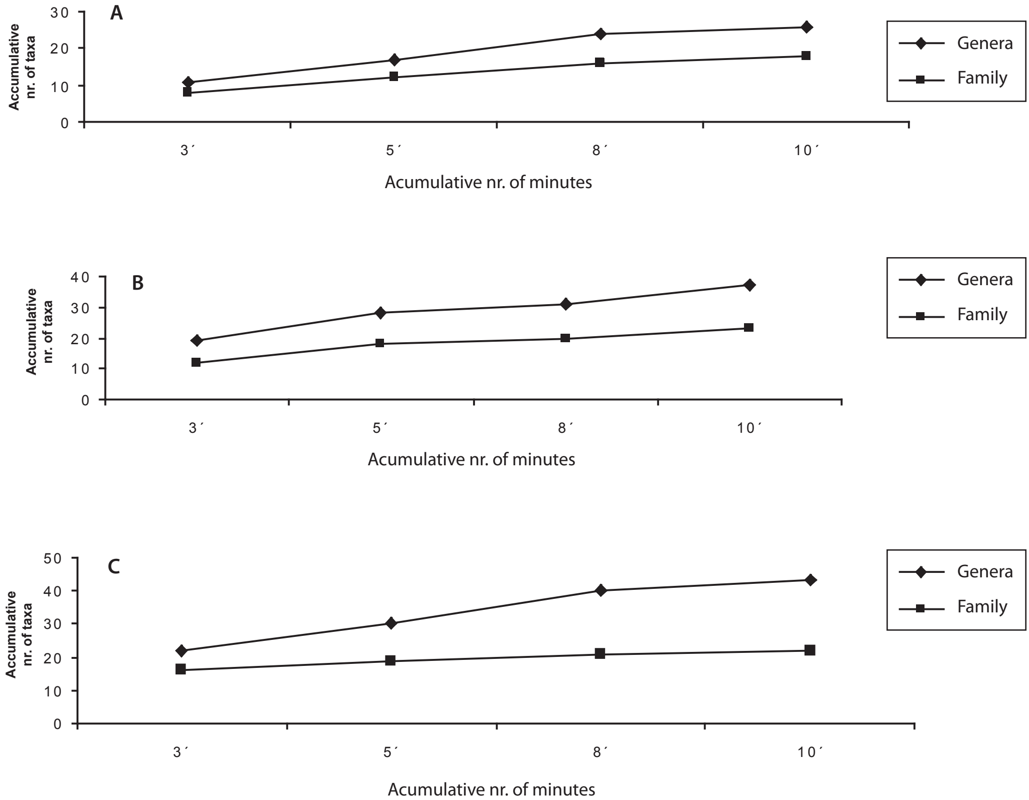

Fig. 5. Accumulative number of genera and families collected with the first method in the dry season in the three rivers: (a) Río Uvita, (b) Río Balso, (c) Río San Lorencito.

2 h collecting time. There is also a difference between leaf packs, since fresh leaves don't house as many aquatic macroinvertebrates as older accumulations. With the second method one realizes very quickly whether an accumulation houses many individuals or not. If only a few individuals are found one has the option of discarding the sample and going on to the next microhabitat, while with the first method a less rich microhabitat is noticed only in the laboratory.

The generally higher abundance and taxa richness with the second method shows that a higher sampling effort is required with the first method. In order to guarantee a more representative sample with the first method, it is necessary to collect a higher number of replicates of the medium time samples (5 or 8 min), rather than increasing the sampling time of a single sample. More investigation is needed to indicate the optimal number of replicates required.

This is also indicated by the species accumulation curves, which showed that new genera and families were still being found after the maximum collecting time regardless of season or river, and therefore more studies are needed to find out when the maximum taxonomic diversity of aquatic macroinvertebrates will be collected. Several authors (Paaby 1993, Flowers and Pringle 1992, Wallace cited in Kricher 1997) mention that the large majority of organisms found in the neotropics are, in contrast to the temperate zones, represented by very few individuals, but in a vast diversity.

The total BMWP-CR index, calculated by using all families found in both methods

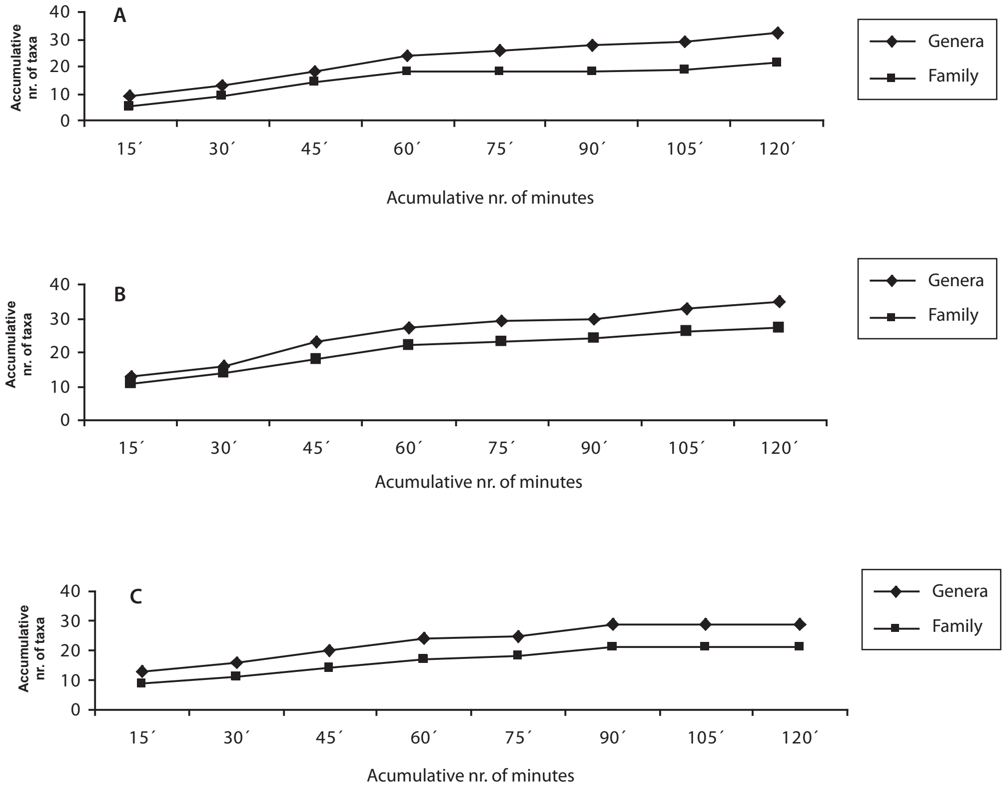

Fig. 6. Accumulative number of genera and families collected with the second method in the dry season in the three rivers: (a) Río Uvita, (b) Río Balso, (c) Río San Lorencito.

combined, shows that a still higher ranking is possible, which could also be seen with the species accumulation curves. Since the highest number of collected families reflects the real water quality of the river, neither of the two methods used in this study fulfills the expectations. Comparing the two methods, the second method always achieves a higher level than the first method, and therefore could be considered to be the more representative one. On the other hand, both methods showed different results in the rainy and the dry season. In the dry season the BMWP-CR index seems to be higher than in the rainy season, and therefore it might make sense to adapt the index or the methodology to each season. Also, it has to be considered that Costa Rica is a very heterogeneous country with significant differences in precipitation depending on elevation and location (Atlantic versus Pacific slopes, and Central Valley), and therefore it might be practical to adapt the BMWP-CR index for the different provinces or watersheds.

Concerning the expenditure of human labor, the first method is more time-consuming since the separation in the laboratory of a single sample can take up to 5 h. With the second method one needs 2 h to collect in the field, but the separation time is omitted so that the second method can be considered the more efficient and more effective method, both in the expenditure of human labor and the results. On the other hand, the results obtained with this methodology could certainly depend on the experience of the person who collects the sample.

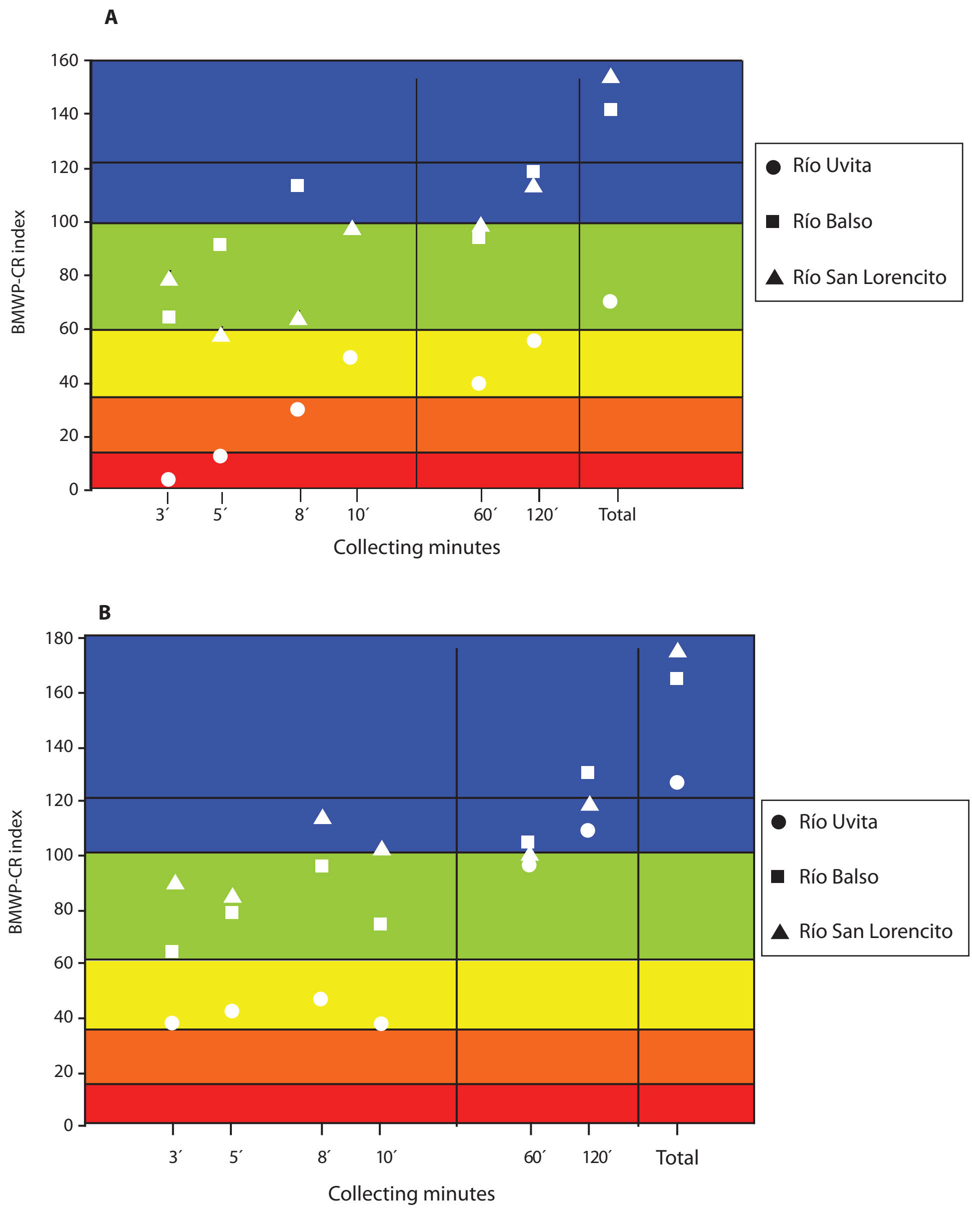

Fig. 7. Values from the BMWP-CR index for each river and method: (a) rainy season, (b) dry season. (Colours represent water quality levels as defined in Table 1).

In summary it can be concluded from this study that the second method, where the individuals were sorted out directly in the field for a time of 120 min, is the one that better represents the taxonomic diversity of a given site, and therefore its water quality. If the first method (recommended: 5 or 8 min) is chosen for biomonitoring, it is necessary to take several replicates. More investigation is needed in order to find out which would be the optimal number of replicates and sampling time to adapt biomonitoring methods used in temperate areas to tropical environments.

ACKNOWLEDGMENTS

We thank Gerardo Umaña Villalobos and Federico Bolaños Vives, for the support with the statistical analysis, Paul Hanson for revising earlier drafts of the manuscript, and the anonymous referees for their comments. The DAAD, Germany, partially supported this work financially. This work is a contribution to the Museo de Zoología, Escuela de Biología, Universidad de Costa Rica.

RESUMEN

En zonas templadas, los macroinvertebrados acuáticos han demostrado de ser una herramienta útil en los estudios de calidad de agua y el biomonitoreo. Recientemente, se han realizado esfuerzos para adaptar estos métodos a los ambientes tropicales; sin embargo, aún existen dudas sobre la metodología y el tiempo de recolecta más adecuados. En el presente trabajo, tres ríos, uno en la provincia de Alajuela y dos en Puntarenas, fueron estudiados con dos métodos y diferentes tiempos de recolecta, tanto en época lluviosa como en época seca. El primer método incluyó la recolecta de materia orgánica e inorgánica por un tiempo fijo (3, 5, 8, 10 min) con un colador. El material fue transferido a un recipiente plástico con alcohol al 70% y los macroinvertebrados fueron separados posteriormente en el laboratorio. Con la segunda metodología, los organismos fueron recolectados directamente desde el colador por un tiempo total de recolecta y separación de 120min y preservados en el campo en alcohol al 70%. Con el fin de obtener curvas de acumulación de especies para este método, se tomaron submuestras cada 15min. El análisis de los resultados mostró que tanto la abundancia como la riqueza taxonómica fueron mayores con el segundo método (de recolecta directa) y una mayor cantidad de géneros pudo ser encontrada con un mayor tiempo de recolecta, aunque no necesariamente una mayor cantidad de individuos. Entre época lluviosa y época seca se observó una diferencia en el número de individuos. Las curvas acumulativas de especies para las muestras tomadas con ambas metodologías mostraron que aún después del máximo tiempo de recolecta se encontraron nuevos géneros y familias, independientemente de la época o del río. Las categorías de calidad de agua obtenidas del índice BMWP-CR varían fuertemente entre tiempos de recolecta y método utilizado. El segundo método de recolecta (directa), siempre reveló una mejor calidad de agua que el mayor tiempo de recolecta indirecta (10min) del primer método. Sin embargo, tampoco llegó al nivel que se obtuvo si se sumaron todas las familias encontradas con ambas metodologías combinadas. Aunque el primer método es el que se utiliza oficialmente en muchos protocolos de biomonitoreo en zonas templadas, estos resultados sugieren que aún es necesario realizar más estudios sobre el tiempo de recolecta y la metodología más adecuada para ríos tropicales.

Palabras clave: biomonitoreo, macroinvertebrados acuáticos, calidad del agua, curva de acumulación de especies, ecología de ríos, Costa Rica.

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