

# **Examination of riverine macro invertebrate riffle assemblages with particular reference to impact of tea plantations**

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## **Abstract**

Freshwater habitats are under threat globally due to human impacts and are relatively little studied in tropical areas. Therefore studies of biodiversity and the effects of human activities are important. In the present study we examined the macro invertebrate diversity in rivers of the East Usambara Mountains, Tanzania. We then focused on the effects of tea plantations as a form of land use in the catchment of our system. We also found that diversity was lower in the Usambara Mountains compared to the reference tropical site in Brazil. Our most salient finding was that tea had a significant effect on river macro invertebrate biodiversity.

## **INTRODUCTION**

There is an increasing realisation that freshwater ecosystems are both diverse and are vulnerable to human impacts (Dudgeon 2006, Junk 2002). Furthermore, freshwaters provide important goods and services of both economic and societal significance such as improvement of water quality and provision of resources (Costanza *et al.* 1997). Riverine habitats are believed to be subject to particularly high levels of risk associated with environmental degradation (Malmqvist and Rundle 2002), and those in the tropics are little studied and less well protected by legislative bodies than those in temperate areas (Thorne and Williams 1997). Only 11% of limnological papers published between 1989 – 1996 concerned tropical rivers, as a result the loss of diversity from these systems is likely underestimated (Malmqvist and Rundle 2002, Thorne and Williams 1997). Therefore the study of tropical riverine habitats is of critical economic and conservational importance.

Threats to rivers may be summarised by direct removal of organisms, ecosystem destruction, physical habitat alteration, and modification of water chemistry (Malmqvist and Rundle 2002). A mechanism for the two latter impacts can often be human land use in the catchment (Moss 1988). We examined 9 upland rivers in the Eastern Arc Usambara Mountains of Tanzania with the aim of investigating the effects of land use on riverine ecology. This area is known to have a high level of species endemism, and is an important area of conservation. The catchment, as described by Bruen (1989), is montane forest interspersed by human land use, at an approximate elevation of 250 –

1400m. Many rivers originate in this area, and contribute to the watershed of the Sigi River which provides water to about 160 000 people, including the city of Tanga. Land use in the river catchment, and its effects on riverine characteristics is therefore important. Tea plantations represent a large component of land use in the East Usambaras, and are particularly prevalent in the highest part of the rivers' catchments. Our main objectives were to evaluate the effect of the presence of tea plantations on the diversity of streams and to compare the general diversity encountered in Usambara streams with other tropical areas. We also aimed to compare our species diversity with 3 rivers situated in the region of Sao Paulo in Brazil. To accomplish these objectives we focused on the macro invertebrate communities in cobbled riffle habitats, following Melo and Froelich (2001).

Invertebrate biodiversity is considered a useful indicator of riverine ecology (Melo and Froelich 2001, Thorne and Williams 1997). Macro invertebrate communities are particularly suitable indicators of the condition of running waters because they are found in most freshwater environments, are relatively easy to sample and show varying degrees of sensitivity to the impacts related to land use, such as agriculture (Boothroyd and Stark 2000). Community indicators suggesting impacts may be compared to the biodiversity and community composition within a control site. Alternatively expected community characteristics may be examined; for instance upland rivers are expected to have a high abundance of shredding organisms and low filter feeder abundance (Abdollah *et al.* 2004, Chapman and Chapman 2003).

We hypothesised that the tea might impact rivers through the following mechanisms: loss of canopy cover, increased physical disturbance (such as trampling), change in chemical properties of water, and increase in sedimentation due to runoff. Other studies have shown that these impacts can alter aquatic macro invertebrate communities (Chapman and Chapman 2003, Thorne and Williams 1997). We expected to observe a reduction in biodiversity and a change in community structure in between tea sites and control rivers. We had no *a priori* expectation about the comparison of diversity between the Usambara streams in our study and those in Brazilian montane and sub-montane forest examined by Melo and Froelich (2001).

## **MATERIALS AND METHODS**

### **Sampling area**

We sampled invertebrates inhabiting stones in riffles of nine different mountainous streams to check whether there is any influence of tea plantation on riverine habitats. All of the streams are

part of Sigi River catchment and are located both inside and in the surroundings of Amani Nature Reserve (ANR) in North West of Tanzania (Latitude: 05°10' S). Moreover, additional data, collected during TBA class exercise 2007, from Emau river were used.

Five control and four tea plantation rivers were selected (Table 1). The first category comprised streams surrounded by forests and undisturbed by human at least one kilometre above sampling site. The latter comprised rivers situated within tea plantations having only small riparian buffer zones (no more than ten meters).

**Table 1. Rivers examined in this study with elevation and location (GPS coordinates)**

River name	Medium altitude	Location South	Location East
Amani stream – control	844	S05.10239	E038.63109
Emau river in Amani - control	797	S05.10303	E038.63144
Goldmine area – control river	965	S05.10409	E038.59728
Kwamkoro – control river	985	S05.15266	E038.60156
Dodwe –tea	1019	S05.08763	E038.60577
Goldmine area – tea	948	S05.10819	E038.59517
Kwamkoro – tea	966	S05.14733	E038.59891
Kwankuya – tea	885	S05.04439	E038.64397

### **Collecting invertebrates**

At each site we randomly selected three riffles over a 500 m reach of the river. In each riffle, seven randomly chosen stones of similar size were collected (within a range to fit into a 10 x 20 cm sampling net). The number of stones was chosen according to a sample curve, following the methodology of Melo and Froellich (2001) and species-sampling curves generated by the TBA 2007 course on the Dodwe and Emau Rivers. Riffle depth was measured after each stone had been taken. Dimensions (length, width, height) of stones were taken to estimate the surface area of each stone (assuming a cubical shape).

Stones were brushed for one minute to remove all animals and, afterwards, all visible invertebrates were sampled. They were identified to the lowest taxonomic level possible, using a key by McCafferty (1998) then identified to morphospecies.

## **Physical data**

At each site we measured a number of physical stream and water chemistry characteristics which may be related to macro invertebrate diversity. We measured dissolved oxygen using a test kit (HI 3810; Hanna), with Azide modification titration method. Dissolved oxygen is crucial for organisms inhabiting riverine habitats (Sutherland 1996). Many species from Ephemeroptera, Trichoptera, and Plecoptera are very sensitive to shortages in dissolved oxygen (McCaffery 1998), so that it might change species abundance in different assemblages (Thorne and Williams 1997). We estimated water flow by measuring the time a plastic sampling jar took to float over a measured riffle length and multiplied this by cross-sectional area (depth x width) to obtain flow rate (Moss 1988). It should be noted that this method only assumes surface flow is representative (Sutherland 1996). Conductivity, pH and redox potential were measured using a Hanna water quality meter. Conductivity depends on electrolyte number and charge. Redox potential measures the ability of water to donate or accept (negative or positive potential) electrons from different substances (Sutherland 1996). Canopy cover was measured with a standard densitometer. Deforestation may decrease canopy cover, reducing the amount of light reaching the water surface, thus change the productivity of habitats and influence community assemblages (Chapman and Chapman 2003). Finally, in each river two samples of particulate matter and bio film covering stones were collected by scraping and washing an area of 16 cm<sup>2</sup> onto filter paper. After 24 hours of drying, all filter papers were weighted and a bio film mass calculated. Bio film consists of both organic and mineral matter, namely: algae, bacteria, sediments and soil particles. This could be used as an indicator of biological coverage and/or sediment load.

## **Data analysis**

Our primary response variables were macro invertebrate species richness, rank abundance, and the EPT index a ratio indicating the relative abundance of disturbance sensitive and tolerant species. We analysed data with ecological and statistical software. Total species richness was estimated for each river using the freeware program EstimateS (Colwell 2006). We report Jackknife 2 estimates of species richness (Smith and van Belle 1984) following previous researchers who showed it to be the most effective metric for tropical stream macro invertebrate communities (Melo Froehlich 2001; Smith and van Belle 1984). We qualitatively compared species rank abundance curves to evaluate patterns of dominance and rarity for each river. The EPT index is an abbreviation for: Ephemeroptera (mayflies), Trichoptera (caddisflies), and Plecoptera (Stoneflies). These are compared to a tolerant taxon, Chironomidae (midges), as a ratio (Chironomidae : EPT; Thorne and Williams 1997).

Statistical analyses were conducted in Minitab and Systat to test for differences in our focal response variables and physical and water chemistry characteristics between control and tea sites. For each comparison we first tested whether data met assumptions of normality and equal variances for parametric tests. Student's t- tests were used when data met assumptions while Mann-Whitney U non-parametric test were used when data were non-normal. Finally, we used least squares linear regression to explore the relationship between physical and water chemistry characteristics and patterns of macro invertebrate diversity .

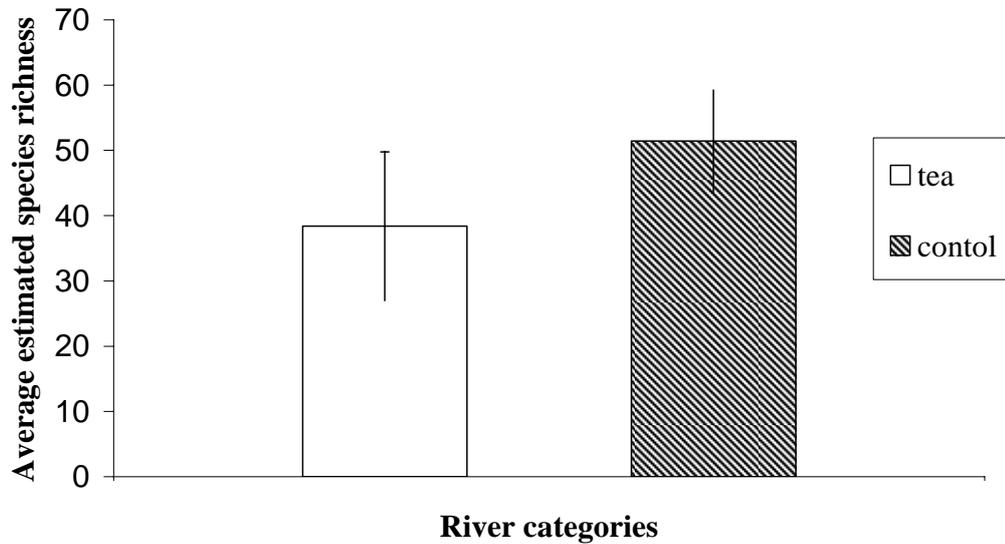
### **Comparison between Usambara and Sao Paulo, Brazil streams**

We compared our rivers with those of Sao Paulo region, Brazil. We examined three rivers from Sao Paulo (Latitude: 22°45 - 24°18 S) which were the Japi, Carmo and Pinda (Melo and Froehlich 2001). They may be considered comparable with our study rivers because they were of a similar order (3<sup>rd</sup> and 4<sup>th</sup> order streams) to those in the present study, and both are situated in tropical moist montane and sub-montane forests (540 – 950 m) and receive similar rainfall (Sao Paulo: 1400 – 1696mm; Amani 2000)

## **RESULTS**

### **Species richness**

The total macro invertebrate species richness was more than 25% lower in tea rivers compared to control rivers (Fig. 1;  $t = 2.380$ ,  $df = 7$ ,  $P = 0.049$ ). Estimated total species richness ranged from 42.05 - 58.38 control sites and 28.93 – 54.91 in tea sites. However only one tea site (Gold-Tea) had estimated species richness comparable to control sites (Table 2).



**Figure 1.** Estimation of species richness of macroinvertebrates in nine rivers of East Usmbra mountains (Jack 2 estimator, EstimateS software)

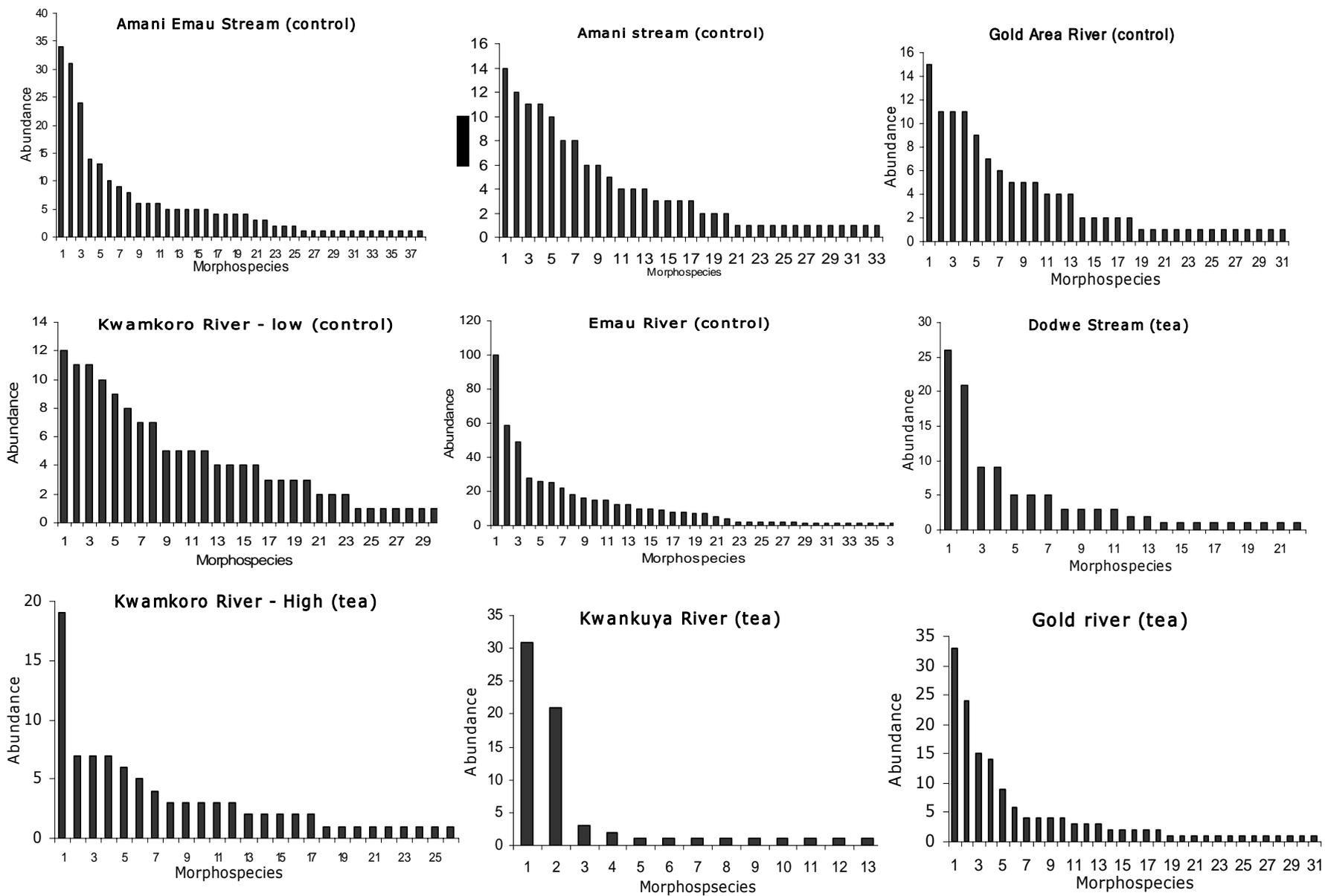
### Species rank abundance curves

The highest number of morphospecies (38) was recorded in the Amani Emau (control) river whilst the lowest was recorded in the Kwankuya (tea) river. Qualitatively, control rivers were typically characterized by greater evenness and a greater number of rare taxa, while tea estate sites were frequently dominated by a few taxa and fewer taxa in total (Figure 2). Generally, Baetidae A and B, Heptageniidae A and B (Ephemeroptera), Hydropsychidae A and Hydropsychidae C (Trichoptera) were found to be dominant in the control river, while Simuliidae A and B, Chironomidae B (Diptera) and Baetidae B (Ephemeroptera) were found to be dominant in the ‘tea’ rivers.

**Table 2. Physical, water chemistry, and species diversity characteristics for nine study streams in the East Usambara Mountains, TZ.**

River	Category	Physical features of habitat							
		Avg. Canopy Cover %	Avg. estimated flow (flow*depth*width)	Avg. DO2 mg/L	Avg. Redox (mV)	Avg. Conductivity (µS)	Avg. pH	Avg. stonearea	Avg. biofilm mass
Amani Emau	Control	89.77	1.13	7.20	242.33	45.33	6.93	691.43	134.00
Kwamkoro Low	Control	97.34	3.04	4.35	186.00	27.00	6.70	550.69	121.00
Gold Area	Control	97.92	2.52	4.00	345.33	46.33	6.77	628.10	267.00
Amani stream	Control	98.44	0.19	6.19	221.67	93.00	7.70	571.29	251.00
Emau upper	Control								
Dodwe	Tea	91.94	1.16	2.78	263.33	37.33	6.17	542.19	383.00
Kwamkoro High	Tea	93.93	1.69	3.55	149.67	28.33	6.60	551.69	118.00
Gold river	Tea	82.93	4.75	3.67	257.00	32.33	7.07	528.76	1037.00
Kwankuya	Tea	97.62	0.51	3.85	87.33	28.00	6.63	462.10	496.00

River	Diversity			
	Observed species	Estimated total species	Abundance	EPT
Amani Emau	38.00	58.38 +/-3.04	227	0.51
Kwamkoro Low	30.00	42.05 +/-1.98	136	0.34
Gold Area	31.00	55.95 +/-3.37	120	0.35
Amani stream	34.00	57+/-2.84	151	0.06
Emau upper		43.9 +/-6.87		0.59
Dodwe	22.00	33.76 +/-2.47	105	1.43
Kwamkoro High	26.00	35.98 +/-2.52	89	0.91
Gold river	31.00	54.91 +/-3.19	149	0.19
Kwankuya	13.00	28.93 +/- 2.98	66	0.33



**Figure 2. Species rank abundance curves for five control and four tea estate rivers in the East Usambara Mountains of Tanzania**

## EPT index

There were no significant differences between the chironomid: EPT ratios in control and tea estate stream (M-W U = 23, df = P = 0.71; Fig. 2). However, there was a trend for tea estate rivers to have higher EPT values, which would indicate that their macroinvertebrate communities were characterized by relatively more chironomids (Table 2).

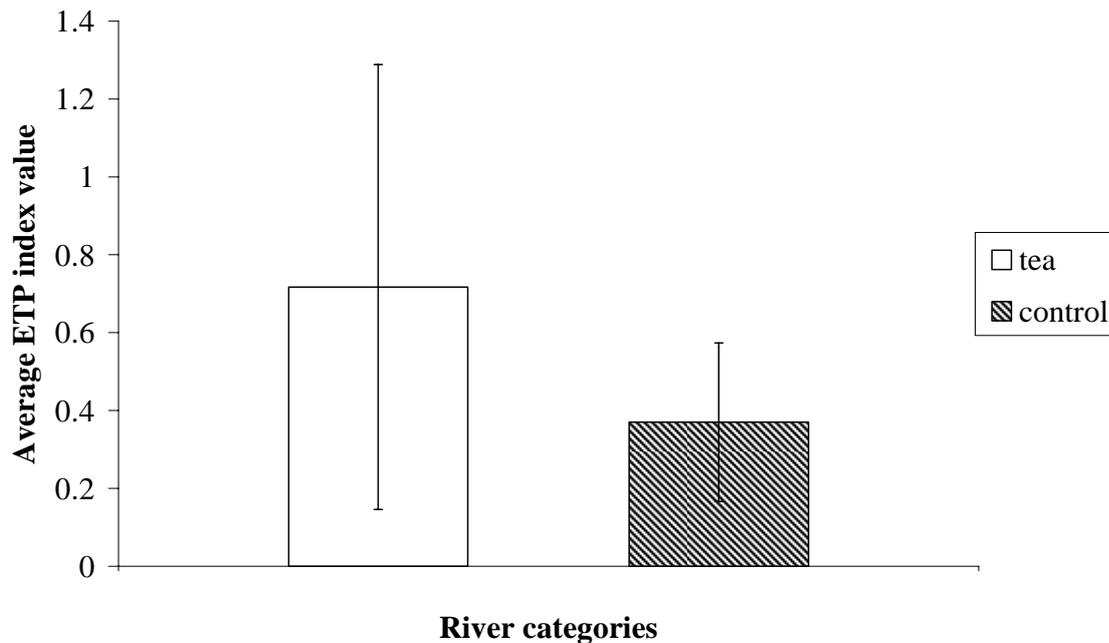


Figure 2. Comparison of average EPT index values for control and tea rivers

## Physical data in relation to biodiversity

Among physical and water chemistry site characteristics, only pH (Mann-Whitney,  $W=114.5$ ,  $p=0.04$ ) and dissolved oxygen value (t-test  $df = 21$ ;  $t$ -value = -3.33;  $P = 0.03$ ) significantly differed between control and tea sites (flow: Mann-Whitney,  $W = 152$ ,  $p = 0.93$  ; redox: t-test,  $t = - 1.64$ ,  $df = 21$ ,  $P = 0.115$ ; Conductivity: Mann-Whitney  $W = 107.5$ ,  $P = 0.02$ , Canopy cover: Mann-Whitney,  $W = 117$   $P = 0.06$ , Biofilm: Mann-Whitney,  $W = 22$   $P = 0.31$ , Stone area: Mann-Whitney,  $W = 6570$   $P = 0.09$ ). We found correlations between dissolved oxygen (Fig. 4.) and pH value and species richness (Fig. 3.). Regression analysis showed that other physical factors are not correlated with species number. No physical factors were correlated with abundance (pH:  $Rsq = 0.11$ ,  $P = 0.06$ ; Estimated flow:  $Rsq = 0.02$ ,  $P = 0.26$ , Canopy cover  $Rsq = 0.00$ ,  $P = 0.96$ , Oxygen  $Rsq = 0.07$ ,  $P = 0.11$ , Conductivity  $Rsq = 0.00$ ,  $P = 0.46$ , Biofilm  $Rsq = 0.00$ ,  $P = 0.74$ , Redox  $Rsq = 0.00$ ,  $P = 0.44$ ).

We used regression analysis to demonstrate the significance of the correlation between species number abundance in control rivers ( $R^2 = 47.4\%$ ,  $P = 0.008$ ). In contrast there is no significant difference in the case of tea rivers ( $R^2 = 1.7\%$ ,  $P = 0.3$ ).

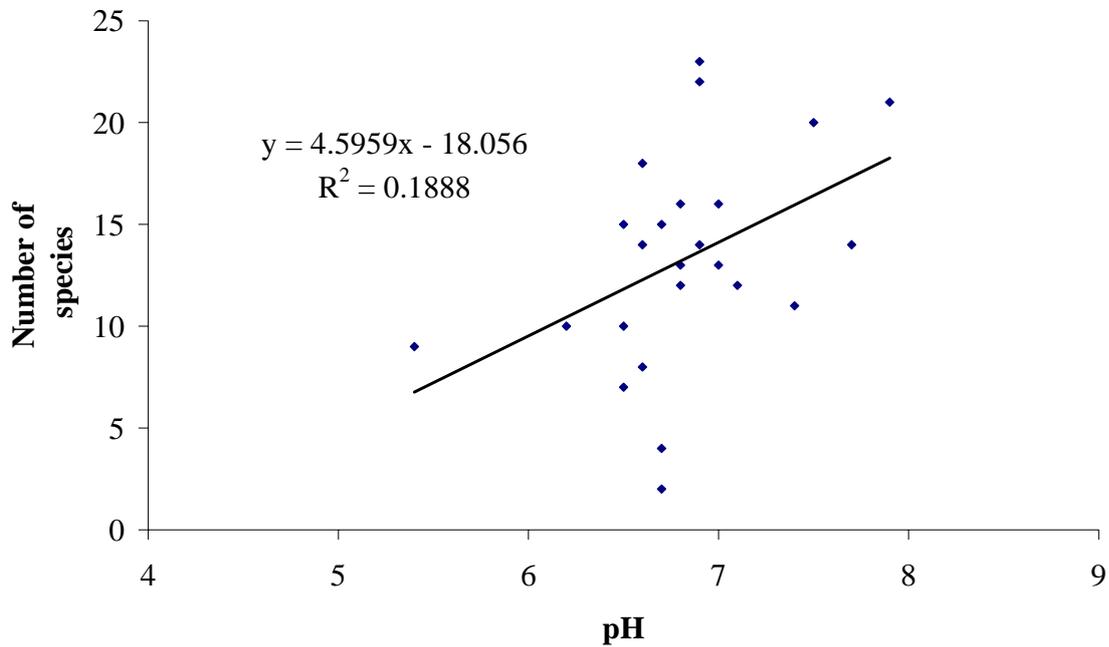


Figure 3. Regression plot of pH against species richness

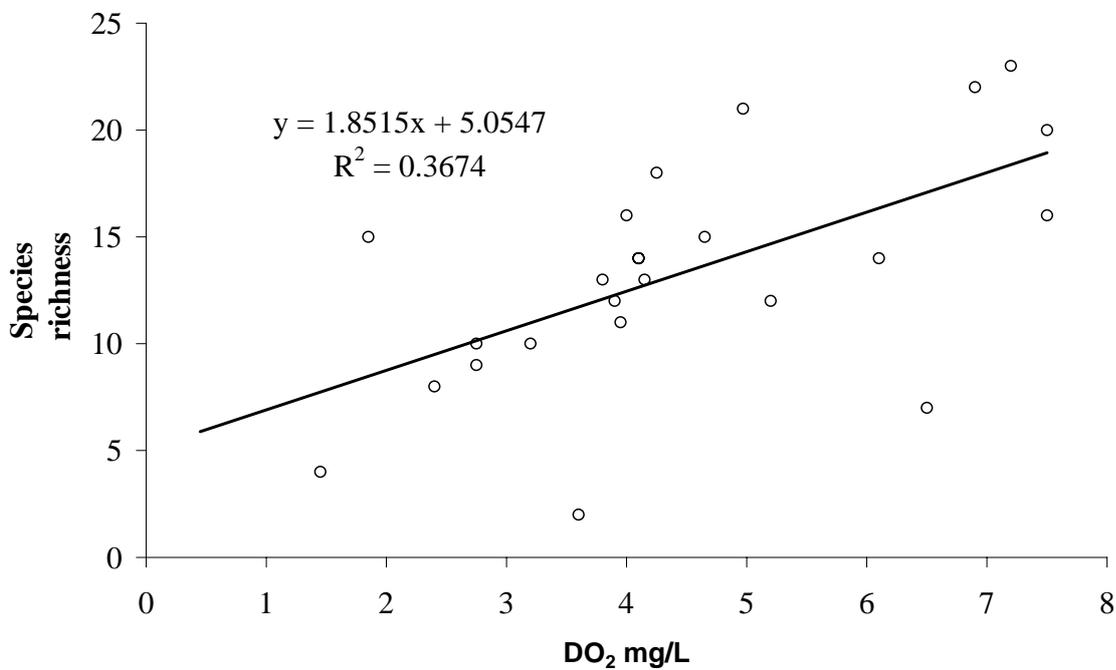


Figure 4. Regression plot of dissolved oxygen against species richness

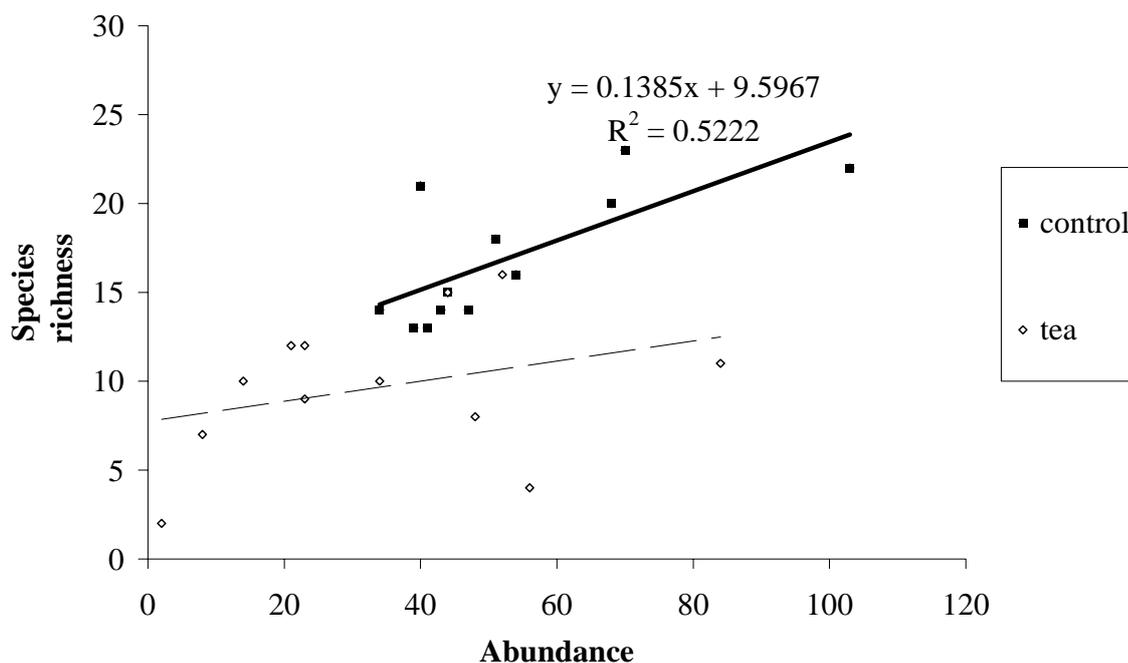


Figure 5. Regression plot of dissolved oxygen against species richness

### Comparison between the Usambara Mountain catchment and Sao Paulo, Brazil

The Brazilian rivers had higher estimated total richness than those from the Usambara Mountains, despite the exclusion of some taxa in the former. Sao Paulo species richness ranged from 63-113 with a mean of 47. Control rivers in the Usambaras (range 42-60; mean 52) were more similar to those of the Sao Paulo site than those surrounded by tea plantations (range 31-56; mean 39). However, even the unimpacted sites were less diverse, particularly considering the exclusion from Melo and Froelich's (2001) analysis of Chironomids and Hydrocarinids.

### DISCUSSION

Species richness values for control and tea rivers were significantly different. This was in accordance with our expectations. We suggest that the predominant reason for this is the increased sediment load entering the river due to increased soil erosion in tea areas.

The only exception to our expectations was the Goldmine site tea river, where species richness was more similar to control rivers. This may have been due to different physical and chemical river parameters in this river. Although it originated in a tea estate and bordered with tea plantation on one side, there was forest on one side of the river. Furthermore pH was high in comparison to other tea and control rivers. This could be important given that pH has a significant positive correlation

with species richness in our study. This parameter has also proved important in determining community structure in other studies (Townsend *et al.* 1983).

We used the EPT index to examine characteristics of the species assemblage. The highest values were recorded in 'tea' rivers. This indicates high proportions of Chironomidae to Ephemeroptera, Trichoptera, Plecoptera. The latter are sensitive to high organic load, whilst Chironomidae are more tolerant. Therefore, a high ratio of Chironomidae is indicative of increased organic pollution (Thorne and Williams 1997). We argue that this is due to increased surface run off from tea plantations in the catchment. Furthermore, a higher number of morphospecies were recorded in the control rivers than the tea rivers. Overall the most of the dominant organisms in the control rivers, reflecting the EPT index results, where Ephemeropterans and Trichopterans and Simuliidae and Chironomidae were the most dominant groups in the tea rivers. The high abundance of species in the control rivers can be attributed to the suitability of these aquatic ecosystems to the survival and growth of a wider range of organisms.

Next, we used regression to examine the association between abundance and species richness in control and tea streams. In control rivers there was a statistically significant, positive correlation between number of specimens and species richness. We did not observe such a relationship in the case of tea rivers. This result might be linked with different species abundance structure of both river categories. If there are few, dominant species in the habitat, adding further specimens will not change species richness so rapidly, as in the case of more diverse habitats. This pattern confirms our interpretation of rank abundance graphs, that diversity in disturbed rivers is lower and that there are fewer and more dominant species. This presents further explanation for the significant difference in species richness between tea and control rivers.

We examined possible drivers of this trend amongst the physical factors. We used a t-test to demonstrate that dissolved oxygen and pH were significantly lower in tea than in control sites. We acknowledge that this is not proof of a causal link between tea and these factors, but an interesting association to explore nevertheless. Regression analysis of pH and dissolved oxygen against species richness showed that 15 % ( $P = 0.034$ ), and 34 % ( $P = 0.002$ ) of species richness were explained by these factors respectively. Certainly pH and dissolved oxygen have been shown to be important determinants of invertebrate assemblages in other studies (Thorne and Williams 1997, Townsend *et al.* 1983).

We suggest that increased runoff from tea plantations, due to reduced water retention, caused increased entry of particulate matter into the river. As a result Biological Oxygen Demand (BOD) in the water would be increased as heterotrophic microorganisms proliferate, thus reducing oxygen concentrations. Factors determining the pH are various and complex (Moss 1988) and the exact reason why tea plantations in the catchment may affect them are beyond our scope. Furthermore, due to the association between many factors, and the lack of experimental manipulation, it is impossible to state the above conclusions with high confidence. Nevertheless, dissolved oxygen and pH may represent components of the mechanism which causes tea sites to have significantly reduced species richness.

### **Species richness comparison between the Usambara Mountain catchment and Sao Paulo, Brazil**

The comparison between rivers of the Sao Paulo area of Brazil and the Usambara rivers examined demonstrated a higher mean and range of diversity in the Brazilian catchment. It is possible that differences could in part be due to human error because of our relative sampling and identification inexperience. Therefore, we are cautious of interpreting this comparison however it gives rise to some interesting speculation. It is possible that invertebrate diversity is higher in Brazil generally than in Tanzania. Furthermore, results appear do not demonstrate the paradigm that biodiversity increases with decreasing latitude over the scale that we examined (Rosenzweig 1995). Indeed, the distance required to observe a latitudinal effect has been the topic of recent discussion within the scientific community with relation to mammals and birds of North America (Orme *et al.* 2006). Aquatic macroinvertebrate diversity over latitudinal clines, and different tropical zones could be of interest in future study, perhaps through meta-analysis of existing data.

### **Summary**

In conclusion, there is a significant effect of the tea in the catchment of rivers in the Usambara area on aquatic invertebrate assemblages. Tea rivers also had lower indicators of riverine quality in their macroinvertebrate makeup, approximated by the EPT index value. Furthermore the presence of a positive correlation between species richness and abundance in control sites (and the absence in tea sites) indicates that control sites have greater diversity, where tea sites are dominated by a small proportion of species. Two possible drivers of the difference between tea and control sites are dissolved oxygen and pH which account for 49% of variance in species richness when combined. It could be the object of further study to examine if these drivers are indeed the most important mechanisms for the proposed effect of tea plantations on riverine macroinvertebrate diversity.

## ACKNOWLEDGEMENTS

Many thanks to all TBA staff for their help and guidance. A particular mention for James's painstaking guidance and late night corrections! Thank you! Also thanks for Moses' help in driving us to far flung tea rivers – much reduced our exertions!

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## Appendix 1

Table 3. List of determined morphospecies

Group	morphospecies
Coleoptera	Coleoptera A
	Coleoptera B
	Dytiscidae
	Elmidae
	Psephinidae
Diptera	Athericidae
	Ceratopogonidae
	Chironomidae a
	Chironomidae b
	Chironomidae c
	Chironomidae d
	Empididae a
	Empididae b
	Empididae c
	Psychodidae (?)
	Simuliidae a
	Simuliidae b
	Simuliidae c
	Tipulidae a
	Tipulidae b
Ephemeroptera	Baetidae a
	Baetidae b
	Baetidae c
	Baetidae d
	Heptagenidae a
	Heptagenidae b
	Heptagenidae c
	Heptagenidae d
	Leptophlebiidae a
	Leptophlebiidae b
	Leptophlebiidae c
	Leptophlebiidae d
	Leptophlebiidae e
Tricorythidae a	
Hemiptera	Hemiptera a
	Naucoridae
	Notonectidae
	Veliidae a
	Veliidae b
Odonata	Libullidae
	Aeshidae
	Calopterygidae

Plecoptera	Perlidae
Trichoptera	Glossomatidae (?)
	Helicopsychidae
	Hydropsychidae a
	Hydropsychidae b
	Hydropsychidae c
	Hydropsychidae d
	Hydroptilidae
	Lepidostomatidae
	Philopotamidae a
	Philopotamidae b
	Philopotamidae c
	Trichoptera ? (Kwankuya)
	Trichoptera NI
Acarina	Hydracarina a
	Hydracarina b
	Hydracarina c
	Hydracarina d
	Hydracarina e
	Hydrocarina f
Gastropoda	Gastropoda a
	Gastropoda b
	Gastropoda c
Turbellaria	Planarian
Decapoda	Crab
Ostracoda	Ostracoda
Hirudinea	leech