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Impact of altitude on spring macroinvertebrates and water quality in South West region of Cameroon

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Abstract – We evaluated the impact of altitude on the biodiversity and water quality of 13 springs located from 82 to 1,189 m a.s.l. on Mount Cameroon. The springs were of good chemical and ecological quality whatever the altitude. The analysis of the physicochemical variables showed low temperature levels (19.50 ± 2.09 °C), high turbidity (13.0 ± 7.17 FTU), and an acceptable mineralization level (324.95 ± 260.0 µS/cm), with high amounts of phosphate nutrients (0.83 ± 0.47 mg/L). We observed a strong seasonal effect, with a decrease in temperature and nutrient content during the dry season whereas pH and dissolved oxygen increased. A total of 10,265 organisms, distributed into 56 families, were collected. They mostly included insects (47.8%), closely followed by Arachnida (34.8%). Only two stygobite taxa were recorded, namely Darwinulidae and Stenasellidae. Total diversity slightly decreased with altitude, especially during the dry season. Despite lower temperature and more oxygen at higher altitudes, diversity, including EPT did not increase. Therefore, African fauna are less sensitive to rising temperature than the faunas of other areas of the world. This result may be explained either by the fact that African species are better adapted to warm, low-oxygen waters than species from other parts of the world, or by the absence of refuges in Mount Cameroon that are home to temperature-sensitive species. Consequently, the impact of climate change on aquatic macroinvertebrates in tropical Africa could be reduced.

Keywords: Biodiversity / Central Africa / elevation gradient / groundwater / temperature

1 Introduction

Climate change, along with the related increase in global temperature, has been reported to have diverse impacts on the physiology, fecundity, growth and biodiversity of animals (Parmesan and Yohe, 2003; Pörtner and Farrell, 2008; IPCC, 2014). Species replacement patterns can be observed in response to climate change (Parmesan and Yohe, 2003), reflecting a latitudinal and altitudinal thermal gradient (Somero, 2010). A powerful approach to predict how global warming will affect species distribution and abundance consists in studying their distribution along an altitudinal gradient (Van der Putten *et al*., 2010).

The distribution of organisms along altitudinal gradients has been studied for many groups of animals in Europe (Bottazzi *et al.* 2011; Von Fumetti *et al*., 2017; Claret and Marmonier, 2019). Although the general pattern is a decrease in taxon richness at higher altitudes, this decrease is not necessarily uniform or similar for all groups of organisms, especially for insects and crustaceans (Rahbek, 1995). However, in tropical freshwaters, especially in Africa, the consequences of temperature change along elevation gradient still remain unclear. Although no strong climate change temperature rise is predicted in tropical freshwaters, uncertainties in the models and spatial heterogeneity are still too high to exclude any risk for tropical regions (Scholze *et al*., 2006; Schuol *et al*., 2008). Concerning the impact of water temperature, studies in Ecuador and Brazil have shown that physicochemical variables such as temperature and oxygen influence macroinvertebrate diversity along an altitudinal gradient (Jacobsen, 2003; Henriques and Nessimian, 2010). The temperature gradient may alter community composition. It may also have a significant impact on animal physiology,

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which may change in order to respond to injuries resulting from temperature variation (Dehedin *et al*., 2013; Cottin *et al*., 2015). This relationship between water temperature and fauna is particularly true in tropical Africa where water temperature ranges are lower than in temperate areas. As a consequence, species living in a more stable environment in Africa might be less adapted to changing temperature than species living in a highly fluctuating environment (Cottin *et al*., 2015). Therefore, it is crucial to improve our knowledge on the effects of elevation-related temperature gradients on those ecosystems.

We are aware that temperature is not the only factor influenced by elevation gradient. Several environmental constraints change with elevation, such as ultraviolet radiations, nutrient availability, riparian vegetation and the chemical composition of the water. It is noteworthy however that they are directly linked to physical elevation above the sea level (*e.g.*, temperature, oxygen) and to local characteristics (*e.g.*, geology, land use) (Körner, 2007). In addition, freshwater ecosystems are facing many challenges such as anthropogenic stressors stemming from the population increase, urbanization, livestock, watering, and commercial agriculture, especially in Africa where water monitoring is absent and waste water treatment is very rare (Lekan, 2001). These activities have led to an increase in pollutants in freshwaters, which affect the water quality and in turn influence the resilience of freshwater ecosystems (Chinche *et al.,* 2019; Zébazé Togouet*et al.,* 2011).

As resurgences of groundwater, springs are good models for studying the impact of altitudinal gradients because they are more stable throughout the year compared to running waters; they harbor both epigean and groundwater fauna exhibiting different sensitivity levels to thermal (Issartel et al., 2005) and chemical (Maazouzi et al., 2016; Marmonier et al., 2018) gradients. Moreover, in countries where less than 50% of the population have access to potable water, many people use groundwater for their daily needs without taking its quality into account (Nola et al.,1998). In a context of climate change and rising water usage (e.g., irrigation, drinking water), the quality of spring waters represents an important challenge for human populations. No study on the effect of altitude and physico-chemical parameters on spring biodiversity has been carried out in Africa altogether, or more particularly in Cameroon.

In this context, the objectives of this study were to answer two questions: does altitudinal gradient have an impact on the spring water quality and the structure of macroinvertebrate assemblages at the interface between groundwater and surface water in Africa? Does this effect change during the wet and dry seasons? To answer these questions, we monitored thirteen springs along an altitudinal gradient on Mount Cameroon (Cameroon) ranging from 82 to 1,189 m a.s.l.

2 Materials and methods

2.1 Study area

Mount Cameroon is one of the highest mountains in Africa, peaking at 4,040 m. It is situated in Fako division (Fig. 1). The division harbors all the major terrestrial ecosystems including rain forest, mountain forest, savanna, semi-arid, arid and freshwater ecosystems with their associated biodiversity (Ngwa et al*.,* 2001). The vegetation shows that 80% of the forest land has been converted to oil palm, rubber and banana plantations by the Cameroon Development Corporation (CDC), and only a few patches of secondary forests are still remaining (Buh Wung, 2009).

The climate of this region is characterized by two main seasons, *i.e.*, the dry season and rainy/wet season. The dry season extends from mid-November to mid-March, with temperature ranging between 27 and 33 °C. The rainy season extends from mid-March to mid-November, with temperature ranging from 19 to 28 °C (Olivry, 1986).

Thirteen springs along an altitudinal gradient ranging from 82 to 1,189 m a.s.l. were sampled seasonally (six in Buea, four in Ekona, and three in Owe). Two sampling campaigns were led at each site at the beginning and at the end of each season in 2017. Samplings were made in November and February for the dry season, and in May and August for the rainy season.

2.2 Water sampling and measurements of physicochemical variables

Water was directly collected at each site in 1,000 mL polyethylene bottles, without producing bubbles. All samples were transported to the laboratory in a cooler containing ice for analysis. The water physicochemical parameters were measured according to the standard methods described by the American Public Health Association (APHA) (1998) and Rodier *et al*. (2009). Water temperature (°C), pH, dissolved oxygen (% sat), electrical conductivity (μS/cm) and total dissolved solids (mg/L) were measured *in situ* using a mercury thermometer, a HACH HQ 11d pH meter, a HACH HQ 30d Flexi oximeter and a HACH HQ 14d portable conductometer, respectively. Salinity (PSU) was measured using a HANNA HI 9829 portable multiparameter. Turbidity (FTU), water color (Pt-Co unit), nitrates (mg/L), nitrites (mg/L), ammonium (mg/L), orthophosphate (mg/L), Ca and Mg Hardness (mg/L) were measured using a HACH DR/2010 spectrophotometer in the laboratory. Bicarbonate (mg/L) was fixed in the field and titrated in the laboratory together with alkalinity (mg/L).

2.3 Fauna sampling and identification

The organisms were collected by passive direct filtration using a sieve (150 μm mesh size, 5 to 8 cm diameter) for one to two hours and the spring water was later filtered at the source. The substrate at the source was disturbed on the same surface (0.25 m^2) with the hand for a period of 1 min after every 15 min to put organisms in suspension with the net facing upstream so as to increase the chances of capturing the organisms. The samples containing organisms were directly fixed in 96° ethanol and brought back to the laboratory for sorting, identification and counting. Sorting and counting were carried out using a Wild M5 binocular magnifying stereomicroscope and an Ivymen R system optical microscope. Taxonomic identification was done based on identification keys published by Tachet *et al*. (2010), Moisan (2010) and Pountougnigni *et al.* (2021).

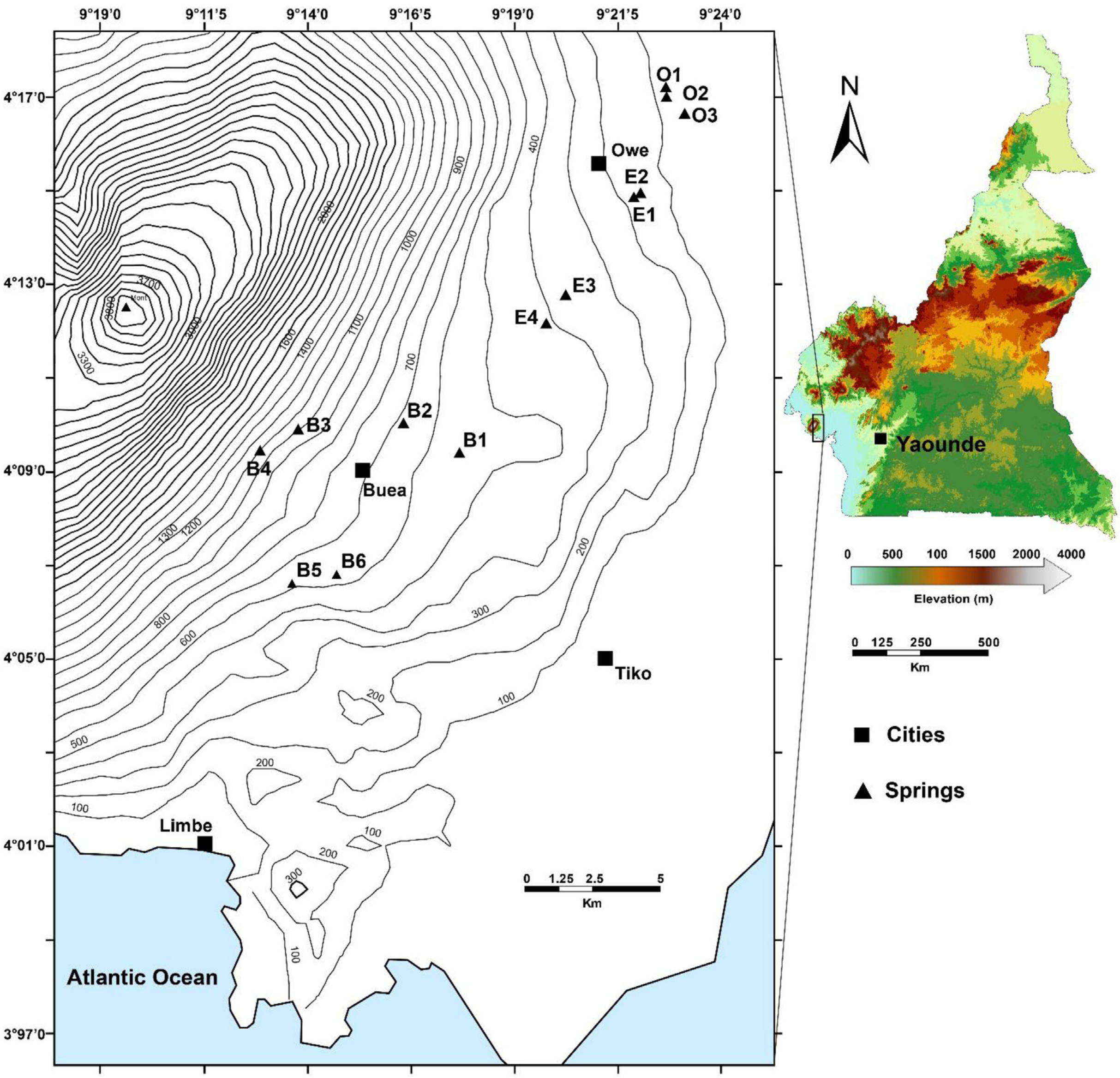


Fig 1. Localization of the sampling sites. Curves indicate 100 m contour lines of Mount Cameroon.

2.4 Data analysis

The correlations between physicochemical parameters were studied using a principal component analysis (PCA) on the basis of the environmental variables measured at each station throughout the study period. PERMANOVAs were used to test the responses of the physicochemical parameters (with Euclidian distance as a similarity measure) or of the faunal assemblages (with Bray-Curtis similarity index) to the factors 'site' and 'season' using Primer 6 software (PRIMER-E). Between-season differences were compared statistically for each environmental parameter using Wilcoxon's matched pairs test. The analysis was carried out using the program Statistica 7 (StatSoft).

Taxonomic richness, richness in Ephemeroptera, Plecoptera, Trichoptera and Coleopteran (EPTC) and Shannon & Weaver's diversity index were calculated using the faunal list of each site and for each sampling date in order to study the effects of site and season. Correlations between each physicochemical variables and faunal taxonomic richness were determined by Spearman correlation tests. A two-way ANOVA was performed to test the variability of the indices across sites and seasons, and Tukey's HSD test was used for pairwise comparisons. The analysis was carried out using the program Statistica 7 (StatSoft).

A distance-based redundancy analysis (db-RDA) was used to detect linear relationships between physicochemical parameters and faunal assemblages (Legendre and Anderson, 1999). Prior to similarity matrix calculations, macroinvertebrate abundance matrix was ln $(x+1)$ transformed to reduce the weight of superabundant taxa upon the analysis. The similarity matrix, calculated using Bray Curtis distances, was used as the input of a principal coordinates analysis (PCoA). The result was a set of principal coordinates representing similarity in a Euclidean space, which is appropriate for analysis using standard RDA. A significant impact of each physicochemical factor was

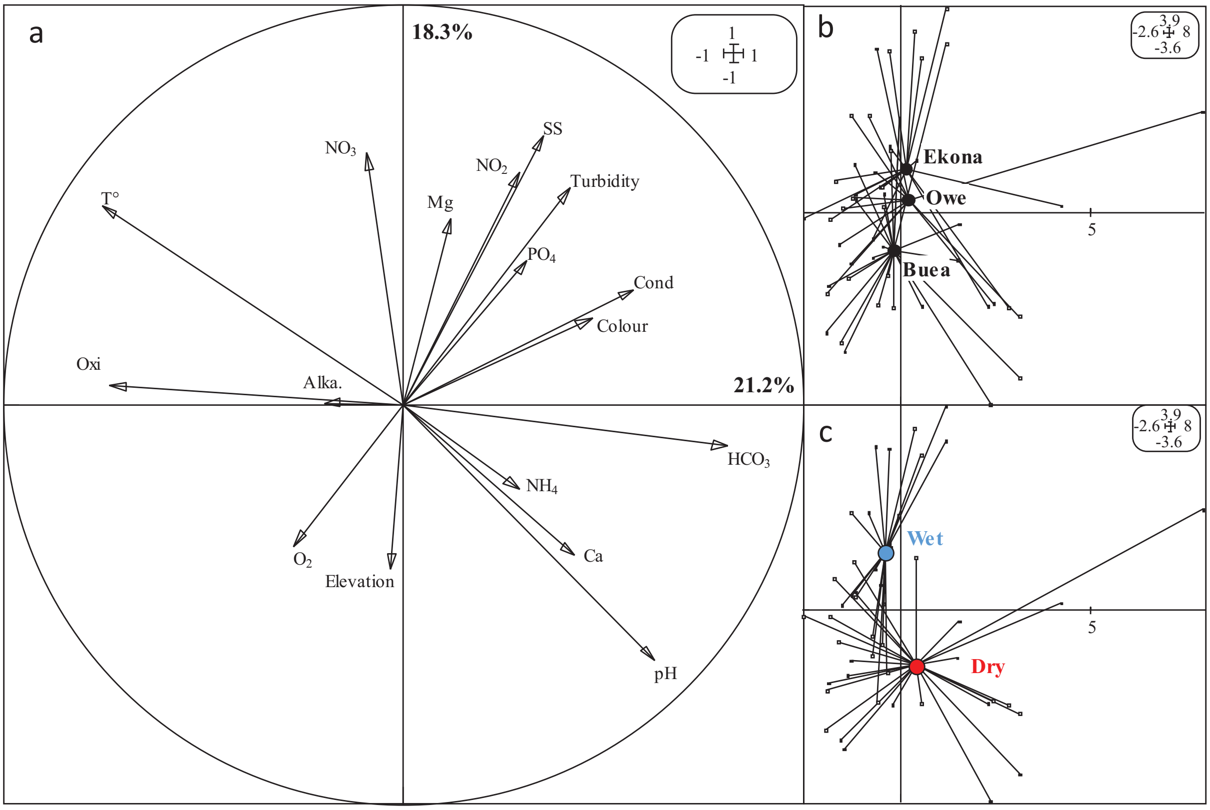


Fig 2. Results of the principal component analysis (PCA) of the environmental parameters of 13 springs. (a) Correlation circle showing correlations among the 17 environmental parameters (Oxi: oxidability, Alka: alkalinity). (b) Distribution of the barycentres of each site (black circles); solid lines link the springs at each season to the corresponding sites. (c) Distribution of the barycentres of each season.

detected by distance-based analysis on linear modelling applied with a backward procedure using the DistLM function in Primer 6 software (PRIMER-E^{TM}).

3 Results

3.1 Variation of physicochemical parameters

The PERMANOVA showed a weak site effect (Fig. 2a, $P = 0.060$) but a strong season effect (Fig. 2b, $P = 0.001$), without any interaction between the two factors ($P = 0.501$). The first two components (PC1 and PC2) of the PCA depicted only 39.5% of the total variance of environmental parameters. PC1 was mainly explained by a high concentration of HCO_3 (18.3%), low temperature (15.7%) and oxidability (14.9%). PC2 was mainly supported by suspended solids (14.5%), pH (13.2%), nitrite (12.7%), and nitrate (10.9%) (Fig. 2a). The three sites were distributed along PC2, with the springs of Buea at the highest altitude (Fig. 2b). The season effect (Fig. 2c) was also depicted along PC2. Elevation was significantly and negatively correlated with temperature ($P = 0.0005$) and electrical conductivity ($P = 0.031$) but was not correlated to any other physico-chemical parameters, even by considering each season independently (*P-values* > 0.106). However, eight physicochemical parameters varied significantly between the dry and wet seasons (Tab. 1). Temperature ($P < 0.001$), nitrite ($P < 0.001$), Mg hardness ($P = 0.006$), salinity ($P = 0.019$), and total hardness ($P = 0.046$) decreased during the dry season, whereas pH ($P < 0.001$), HCO_3 ($P < 0.001$) and O_2 ($P = 0.002$) increased.

3.2 Biodiversity in the studied springs

A total of 10,264 organisms (6,631 in the wet season and 3,634 in the dry season) were collected throughout the sampling period, distributed into 11 classes, and 56 families. Insects were in the highest numbers (4,904; 47.8%), followed by Arachnida (3,568; 34.8%) (Fig. 3). Only two families of groundwater invertebrates were found at Buea (Darwinulidae) and Owe (Stenasellidae).

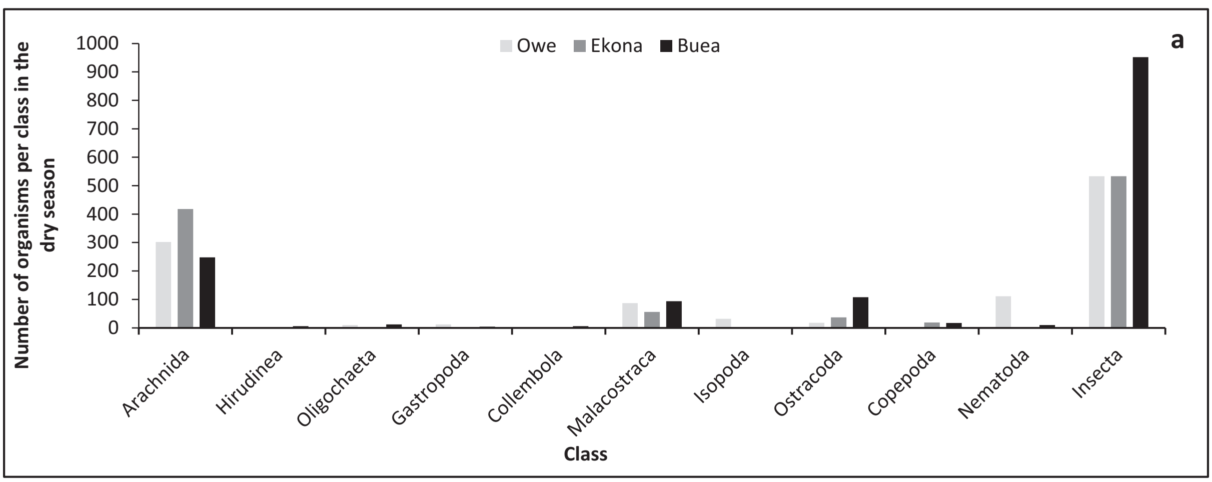
PERMANOVA did not highlight a strong site effect ($P = 0.059$) or any season effect ($P = 0.184$) on the invertebrate community composition. Taxonomic diversity (Fig. 4a) ranged from 1 at spring B2 to 19 at spring O2 and changed significantly between sites ($F_{2,46} = 5.7$, $P = 0.006$), but remained similar between seasons ($F_{1,46} = 0.028$, $P = 0.867$). However, the Shannon diversity index (Fig. 4b) significantly varied between sites ($F_{2,46} = 3.49$, $P = 0.039$) and increased during the wet season ($F_{1,46} = 4.84$, $P = 0.033$).

3.3 Relationships between macroinvertebrate communities and altitude

Taxonomic richness and Shannon diversity or EPTC richness decreased significantly ($P = 0.213$, $P = 0.324$ and

Table 1. Mean values (± SD) of environmental parameters during the dry and wet seasons in Owe, Ekona and Buea.

	Buea		Ekona		Owe	
Parameters	Dry	Wet	Dry	Wet	Dry	Wet
Altitude (m)	789 ± 240	789 ± 240	274 ± 138	274 ± 138	91 ± 7	91 ± 7
Temperature (°C)	20.2 ± 1.6	17.9 ± 3.1	22.5 ± 0.4	20.1 ± 1.8	22.9 ± 0.4	15.2 ± 0.1
Susp. solids (mg/L)	7.2 ± 2.4	10.6 ± 5.8	9.6 ± 4.8	12.8 ± 5.2	7.7 ± 5.5	11.8 ± 2.1
Turbidity (FTU)	9.7 ± 4.9	16.3 ± 8	15 ± 5.9	16.9 ± 6.3	14.7 ± 5.8	11.7 ± 3.3
Elec. cond. (μS/cm)	194 ± 77	456 ± 404	300 ± 20	300 ± 22	440 ± 399	179 ± 48
Salinity (PSU)	0.07 ± 0.026	0.1 ± 0.05	0.15 ± 0.01	0.15 ± 0.01	0.07 ± 0.04	0.08 ± 0.03
pH (CU)	7.5 ± 0.3	6.6 ± 0.2	7 ± 0.4	6.3 ± 0.1	7.5 ± 0.1	6.6 ± 0
Dissolved O_2 (% sat)	74.5 ± 5.9	61.6 ± 1.8	70.6 ± 8.5	61.2 ± 3.3	70.6 ± 3.8	62.7 ± 1.9
Dissolved CO_2 (mg/L)	3.7 ± 2.0	0.3 ± 0.1	10.5 ± 5.4	0.4 ± 0.1	9.3 ± 7.9	0.2 ± 0.1
Nitrate (mg/L)	0.29 ± 0.18	0.48 ± 0.44	0.4 ± 0.27	0.96 ± 0.89	0.4 ± 0.09	0.83 ± 0.38
Nitrite (mg/L)	0.005 ± 0.01	0.032 ± 0.02	0.007 ± 0.01	0.032 ± 0.03	0.015 ± 0.01	0.043 ± 0.04
Ammonium (mg/L)	0.01 ± 0.004	0.003 ± 0.004	0.14 ± 0.39	0.003 ± 0.005	0.37 ± 0.22	0.004 ± 0.006
Orthophosphates (mg/L)	0.57 ± 0.19	1.1 ± 0.54	1.3 ± 0.97	1.85 ± 0.38	0.41 ± 0.06	0.5 ± 0.14
Alkalinity (mg/L)	5.7 ± 2.1	5.2 ± 1.2	5 ± 1.4	7.5 ± 5.3	4.7 ± 2.1	6.3 ± 3.2
Total hardness (mg/L)	41 ± 11	72 ± 49	35 ± 18	163 ± 121	64 ± 8	79 ± 72



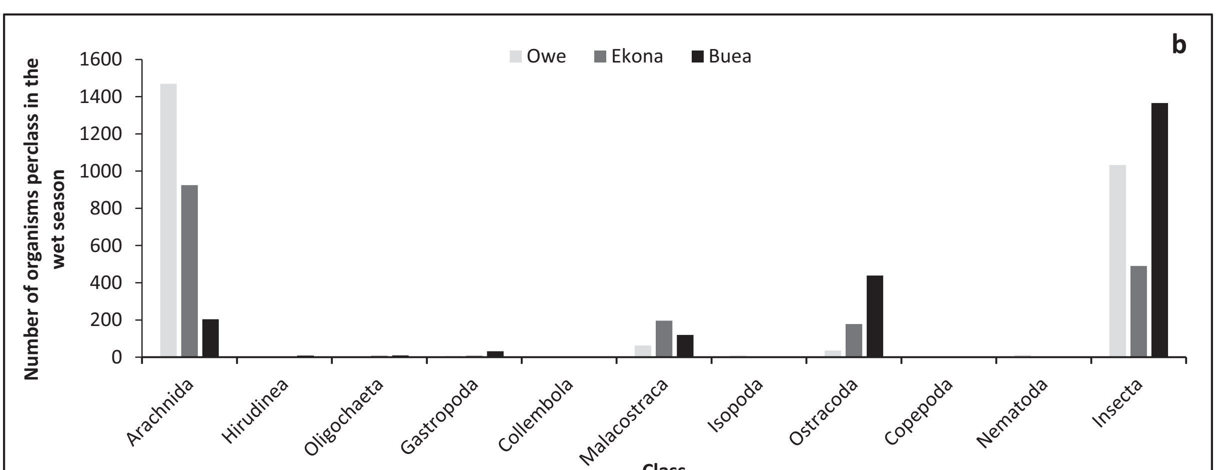


Fig 3. Distribution of the different classes of macroinvertebrates collected in springs in the dry season (a) and the wet season (b).

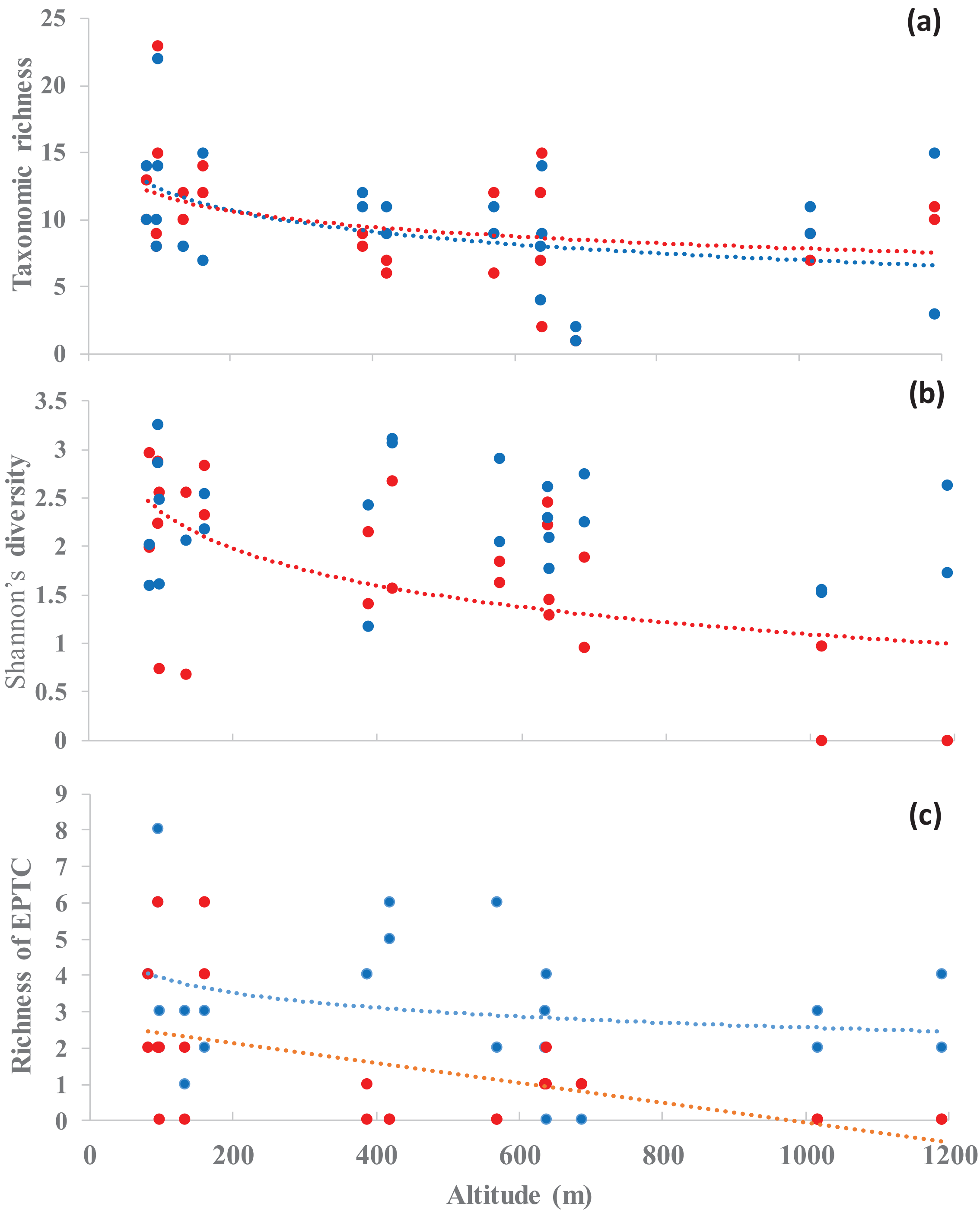


Fig. 4. Altitude-dependent variation of species richness during the wet (blue dots) and dry (red dots) seasons (a), of Shannon & Weaver's diversity index for seasons (b), and richness of EPTC (c) throughout the study period. Dotted lines indicated significant Pearson correlations ($P < 0.05$).

$P = 0.224$, respectively) with increasing altitude (Figs. 4a, b, c). However, the decrease of the Shannon diversity index and EPTC richness were much sharper during the dry season (Figs. 4b and 4c, respectively).

Four families (Cyprididae, Psychodidae, Simulidae and Tipulidae) were positively correlated with altitude (Tab. 2), while five others were negatively correlated (Baetidae, Coenagrionidae, Limnesiidae, Stenasellidae, and Tetragnathidae).

The first two axes of the dbRDA showed that the physicochemical parameters explained almost 31% of the fauna distribution (Fig. 5). Among these parameters, only altitude ($P = 0.001$) and water temperature ($P = 0.05$) were significantly correlated with the faunal assemblages.

Table 2. Significant Pearson's correlations (*r*) coefficient of determination (R^2) between altitude and abundance of macroinvertebrate families.

Family	*r*	R^2	*P*
Baetidae	−0.635	0.43	0.007
Coenogrionidae	−0.467	0.28	0.031
Cyprididae	0.556	0.34	0.018
Limnesiidae	−0.635	0.43	0.007
Psychodidae	0.581	0.34	0.018
Simulidae	0.616	0.38	0.012
Stenasellidae	−0.358	0.23	0.049
Tetragnathidae	−0.433	0.25	0.041
Tipulidae	0.571	0.33	0.020

4 Discussion

4.1 Physicochemical characteristics of the studied springs

The overall water quality of the springs was good in all sites and ranged within the standards of the World Health Organization (WHO, 2017) for almost all parameters and all over the year, even though a few values tended to increase during the rainy season. However, some parameters exceeded suitable limits. Turbidity values (13.8 ± 10 FTU) were higher than the recommended 5 FTU. These high values could be due to (1) the open nature of most of the springs, (2) the presence of trees, herbs and shrubs whose leaves can fall in the springs, and (3) the high temperature that promoted the development of phytoplankton. Turbidity also tended to increase by 30% during the rainy season, highlighting significant runoff caused by rainfall (Ioryue *et al.*, 2018). The average concentration of orthophosphates was high in all springs, especially at Ekona ($1.58 \pm 1.6\,mg.L^{-1}$). These very high values were also associated to strong seasonal variability; a peak usually occurred in May, the first sampling time-point in the rainy season, characterized by strong modifications of the spring environment resulting from the dry-to-wet season transition. In this part of Africa, groundwaters generally contain high amounts of phosphorus, partly due to geology (Nana Nkemegni *et al.*, 2015). However, the strong seasonal variability was also probably partly related to human activities and runoff following heavy rains. The sampling points were close to farmlands where cocoa, plantain and other crops were grown. Zébazé Togouet *et al.* (2011) demonstrated that the high percentages of orthophosphates mostly came from agricultural fertilizers and fecal pollution. They could also result from the daily activities carried out in most of these springs, e.g., bathing or washing of clothes, as all these activities require detergents (Behailu *et al.*, 2018).

4.2 Spring biodiversity

Springs can be considered as ecotones, which are characterized by a mixture of groundwater and surface water species (Cantonati *et al.*, 2020). Natural springs host a variety of species, including taxa that dwell exclusively in the spring mouth – known as crenobionts (Cantonati *et al.*, 2012) –, generalist species that colonise the spring from the surface water (Bottazzi *et al.*, 2011), and others that colonise it from the underlying aquifer (Stoch *et al.*, 2016). The total numbers of taxa sampled in the springs of Mount Cameroon (2 to 30) were relatively low, and the highest mean taxonomic richness (19 ± 5.7) was recorded in spring O2 near Owe. This relatively low diversity stood within a similar range as mountainous springs in Europe (Pešić *et al.*, 2016; Claret and Marmonier, 2019; Pascual *et al.*, 2020), but remained lower than diversity in springs of the volcanic Taranaki mountains in New-Zealand (around 37 taxa in Barquín and Death, 2006). Our study confirms that diversity in springs is high in Africa, especially as our level of identification (mainly at the family level) was higher than in the previously cited studies. The invertebrate community was largely dominated by epigean species, and only two families of stygobite invertebrates were found. The highest number of epigean organisms recorded in the springs could be due to their open nature and to the presence of a water bed during both the rainy and dry seasons which favored the establishment of epigean benthic macroinvertebrates. A previous study on macroinvertebrate from wells in Cameroon (Nana Nkemigni *et al.*, 2015) already highlighted that open wells are colonized by a more diverse fauna, mainly epigean. Taxonomic groups may vary in their sensitivity to certain physicochemical variables and groundwater inputs (Martin and Brunke, 2012; Cantonati *et al.*, 2020). Differing sensitivity levels might explain the dominance of insects in freshwater, slightly acidic volcanic springs (Glazier, 1991). Very high proportions of aquatic insects in acidic tropical streams are widely observed in this part of Africa (Zébazé *et al.*, 2009; Fugère *et al.*, 2018; Ngoay-Kossy *et al.*, 2018) compared with temperate European (Martin and Brunke, 2012; Claret and Marmonier, 2019) and North-American (Glazier, 1991) springs with higher ionic concentration (mainly Ca and Mg) where the proportions of crustaceans and molluscs can be much higher (Gibert and Culver, 2009). In our study, only a few groundwater crustaceans belonging to the families Darwinulidae (Ostracoda) and Stenasellidae (Isopoda) were collected. Darwinulidae were sampled at one site near Buea, whereas Stenasellidae were found in springs near Owe. Previous studies in this part of Cameroon already highlighted very low proportion of groundwater invertebrates, even in wells (Nana Nkemegni *et al.*, 2015; Chinche *et al.*, 2019). The very low ionic concentrations in groundwater may explain on

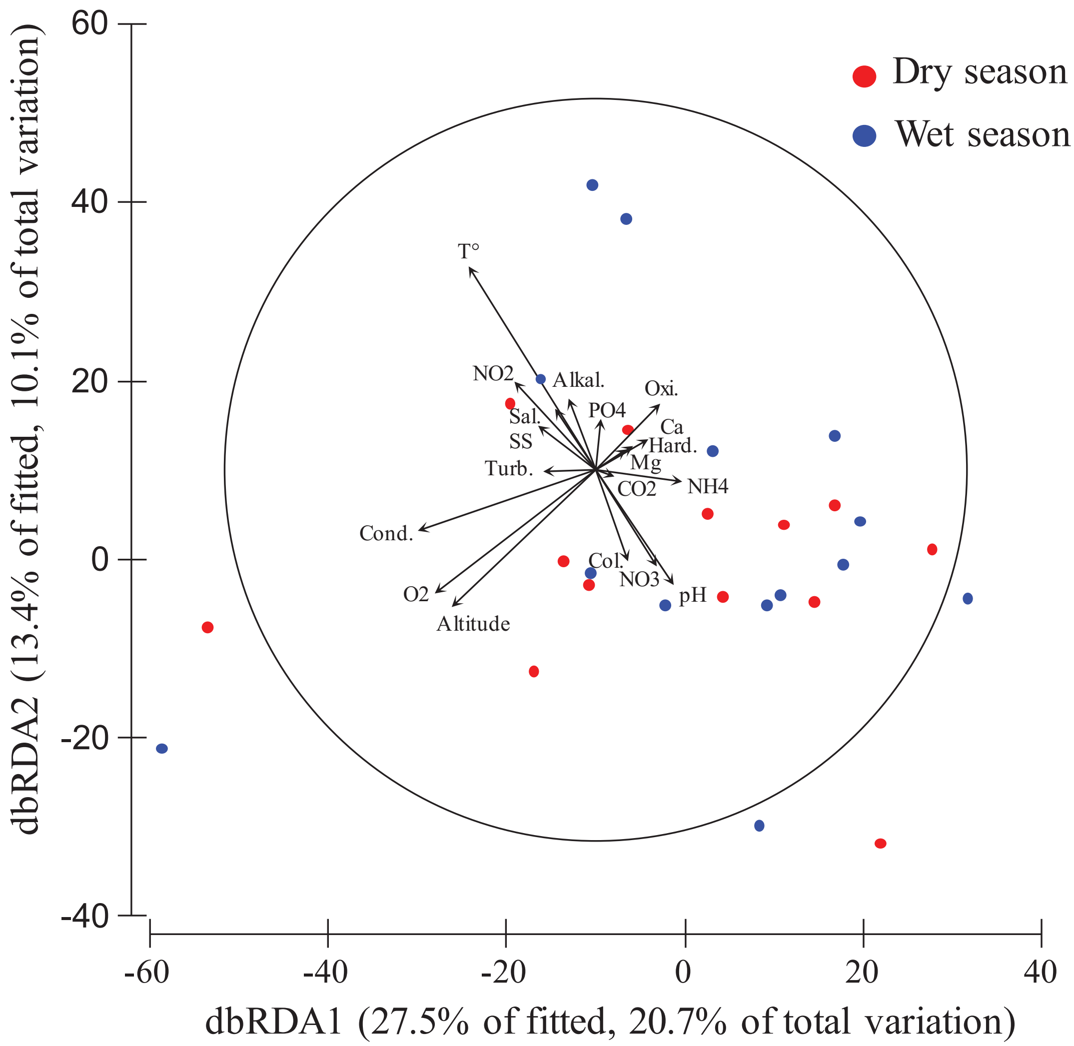


Fig 5. Distance-based redundancy analysis (dbRDA) plot of Distance-based linear modeling (DistLM) results in 2-dimensional space for environmental variables and macroinvertebrate community in dry and wet seasons. The length and direction of the vectors represent the strength and direction of the relationship (Alkal., alkalinity; Col., water color; Cond., electrical conductivity; Hard., hardness; Oxy., oxydability; Turb., turbidity).

one side the lack of groundwater crustacean representing almost a half of groundwater species worldwide (Gibert and Culver, 2009) and, on another side, the fractured nature of aquifers of Mount Cameroon could explain the strong spatial heterogeneity between springs even very close from each other (Pountougnigni *et al*., 2021). The absence of groundwater invertebrates in springs is common and can be explained by their high sensitivity to predation by epigean predators in surface waters (Manenti and Pezzoli, 2019) or by their sensitivity to ultra-violet light (Manenti and Barzaghi, 2021). Another possibility is an unsuited sampling effort to catch groundwater fauna. The abundance of groundwater invertebrates greatly fluctuates over time (Lou and Bloomfield, 2012), even at a daily scale (Alqaragholi *et al*., 2021) and oven locally (Pountougnigni *et al*., 2021). Our sampling methodology with only four samplings a year was likely not sufficient for groundwater fauna.

The overall taxonomic richness of the springs remained stable across the seasons, and faunal assemblages were not affected by the season. This result may be explained by the combination of two factors: (1) our study sites were located in a tropical region with lower seasonal variation than in temperate regions, and (2) most springs had greater physical and chemical stability than higher-order streams (Glazier, 1991). Nana Nkemegni *et al.* (2015) found low but significant seasonal variation in wells closed to our study area with a decrease in diversity during the wet season. However, faunal assemblages in wells strongly differ from assemblages in springs, where diversity is much higher and the proportion of crustaceans – the most affected taxon in Nana Nkemegni's study – is much lower.

4.3 Relationship between altitude, physicochemical parameters and biodiversity

The altitudinal gradient of the springs along Mount Cameroon went along with relatively few significant changes in physicochemical parameters; changes were season-dependent. Only salinity was negatively correlated with altitude throughout the year, and other varying factors (temperature, pH and oxidability) were only correlated with altitude during the rainy season. The decrease in salinity may be explained by the fact that saline water influences (*e.g.*, sea sprays, saline water intrusion in coastal aquifers) are greater at low altitudes and close to the sea. Gonfiantini *et al.* (2001) observed humid air masses coming from the ocean that climbed up along the slopes of Mount Cameroon and produced rainfall that decreased with increasing altitude and distance from the sea. Moreover, the contribution of groundwater to the springs decreases with altitude, while the proportion of rainwater – which is less salted – increases (Abass *et al*., 2017). A decrease of the water ionic content along an altitudinal gradient has already been observed (e.g., Von Fumetti *et al*., 2017; Claret and Marmonier, 2019). Even if other factors such as ionic composition (Ca and Mg hardness) did not highlight a similar dilution pattern, their low concentrations in this volcanic area and the strong correlation between site variability may explain the lack of statistical differences. The temperature decrease in relation to altitude is largely documented under temperate climate in European (Claret and Marmonier, 2019) and North American springs (Schenk *et al*., 2018). Similar patterns are also observed in rainwater in tropical regions on Mount Cameroon and also in South America (Gonfiantini *et al*., 2001). However, this expected pattern was only partially validated in our study site because the decrease in temperature with altitude was only observed during the rainy season. The higher variation in temperature during the rainy season is congruent with the previous hypothesis of an increased proportion of rainwater in spring water. The rainwater temperature is more sensitive than groundwater inputs to air temperature decrease with altitude because groundwater inputs are more thermally stable over time (Kaandorp *et al*., 2019). Contrary to salinity and temperature, the pH of spring water was positively correlated with altitude. We do not have a clear explanation for such a gradient, which could be explained by many geological, physical or biological factors. However, the range of variation was tiny (less than +0.3 pH unit for 1,000 m of altitude) compared with a seasonal variation of ± 0.85.

Contrary to physicochemical parameters, faunal assemblages reacted strongly to the altitudinal gradient. Diversity is supposed to decrease along elevation gradients because of climatic constraints (temperature, duration of sub-zero temperatures) (Claret and Marmonier, 2019; Pascual *et al*., 2020). This impact generally results from the exclusion of species on the basis of their biological and ecophysiological characteristics (Milner *et al*., 2001). We observed a similar trend in the springs located on the slopes of Mount Cameroon, with a 50% decrease of taxonomic richness along the 1,200 m altitudinal gradient. Insects generally dominated at high altitudes while water mites dominated at low altitudes. The dominance of water mites at low altitudes can be explained by several factors, other than their thermal preferences (Di-Sabatino *et al*., 2000). Spring substrates also strongly affect species composition (von Fumetti *et al*., 2017). The springs of Mount Cameroon have relatively similar substrate compositions and are largely dominated by organic substrates, favourable to water mites (von Fumetti *et al*., 2017). For the other groups, the abundances of most insect and crustacean groups decreased along the altitudinal gradient (e.g., EPTC, Gastropoda or Isopoda) or remained similar. Finally, only 4 families increased with altitude, *i.e.*, three Dipteran families (Psychodidae, Simulidae and Tipulidae) and one Ostracod family (Cyprididae). These families are characterized by high dispersal capacities and their tolerance to dryness, which is most frequent in altitudinal springs (Amundrud and Srivastava, 2015). Altitude also had a negative effect on groundwater taxa, which were found only at altitudes lower than 600 m a.s.l.

5 Conclusion

The springs of Mount Cameroon are of good chemical and ecological quality, whatever the altitude. This result is important because water from these springs is used by local people for different purposes, especially as drinking water, and the water is used without prior treatment. Biodiversity decreased with altitude, as observed in temperate climates around the world. Despite lower temperature and more oxygen at higher altitudes, biodiversity did not increase. Therefore, the African fauna reacts similarly to the faunas of other areas in the world. However, the lack of knowledge on African invertebrates precluded identification at a lower taxonomic level than the family level of typical crenal species in higher-altitude springs. Surprisingly enough, EPT richness did not increase at high altitudes compared to other insects, perhaps because of continuous dry weather conditions that promote short-life species like Dipterans or species able to withstand dryness like Cyprididae. African species appear to be well adapted to warm and poorly oxygenated water compared to species in other parts of the world.

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