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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Full Length Research Paper

Study of the influence of environmental factors on the occurrence of *Balantidium coli* cysts in an urban aquatic system in Cameroon

AJEAGAH Gideon A.* and MOUSSIMA YAKA Diane A.

Laboratory of Hydrobiology and Environment, Faculty of Science, University of Yaoundé I, Cameroon.

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***Balantidium coli* is an enteropathogenic cosmopolitan ciliate which causes balantidiasis in humans. There is a high interest in studying it's occurrence in developing countries because of the vulnerability of the population to infectious diseases. The present study was carried out in order to characterise the cysts of the parasite and to evaluate the influence of environmental variables on their dispersal in an urban stream which is being exploited by the population to accomplish urban agriculture, industrial and domestic activities. The presence of round (825 ± 610 cysts/L) and oval shaped cysts (246 ± 300 cysts/L), whose size varies between 30 and 75 µm was noted. A canonical correspondence analysis shows that the lowest density of the cysts and a relatively low concentration of the ecological indicators of organic pollution are observed upstream (E1) and down stream (E_5). High abundance of cyst of *Balantidium coli* was observed in stations located along a piggery effluent associated with a major market. The similitude index of Bray-Curtis shows an 84% of resemblance between E_2, E_4 which are located midstream and very close to a populated urban area. Higher densities of cyst are registered during the short rainy season (207±213 cysts/L).**

Key words: *Balantidium coli*, cyst, distribution, transmission, urban stream, Yaoundé.

INTRODUCTION

An infectious disease constitutes throughout the world, an enormous threat to the populations (Nozais, 1998; Ajeagah et al., 2010). It is closely related to the use of water contaminated with human and animal faeces as presented by the United Nations Economic Commission for Europe (UNECE) and the World Health Organisation (WHO), 1999. Among the waterborne diarrheal diseases of tropical concern, we can mention Balantidiasis. This disease can be fatal in developing countries due to malnutrition, surinfestation and precarious health conditions of the population (Schuster and Ramirez-Avila, 2008). It is caused by ciliated protozoa known as *Balantidium coli*. This is the only pathogenic ciliated protozoa that have been recognized to infect humans and

*Corresponding author E-mail: ajeagahg@yahoo.com.

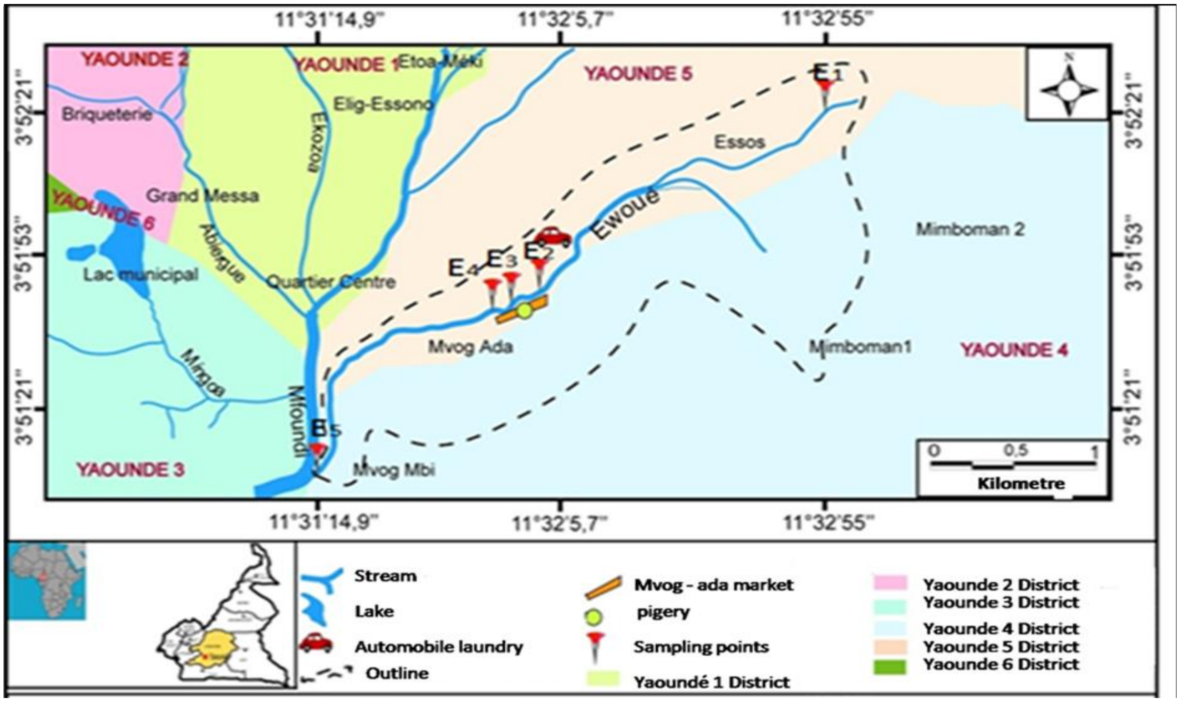

Figure 1. Map of the Ewoue River Basin indicating sampling points.

non-human primates. It is primarily transmitted by the faecal-oral transit mechanism. The cyst is ingested with contaminated food or water then released in the colon and jejunum, a trophozoite which is more or less pathogenic after excystation. Its virulence depends on several factors, such as the strain, the dose in the body, and the host susceptibility (Levine, 1961). Clinical cases could therefore be classified as asymptomatic where the host, *B. coli* reservoir ensures a manifestation of the disease which is characterized by mucous and bloody diarrhoea (Vasquez et al., 1999; Yazar et al., 2004.). In this case, the trophozoite secretes an enzyme called hyaluronidase, which digest blood vessels and lymphatic tissue. The ulcerations that result open the way to the general cavity, thereby increasing the health risk of the victim. According to Schuster and Ramirez-Avila (2008), this may cause infections to the liver and lungs. Cases of uterine infections, virginities and cystitis due to *B. coli* have also been reported. The best way to protect the population against *B. coli* is to avoid contamination of food with faeces of pigs (which is the main reservoir host of the pathogen) and humans. Otherwise in case of infection, taking tetracycline or metronidazole at recommended doses are considered as preliminary treatments of the disease (Schuster and Ramirez-Avila, 2008). Despite its significant health interest in the sub-Saharan countries, the epidemiology of this parasite has been less studied. No study has been carried out on the presence of the cysts of this pathogen in the aquatic environment, as well as the mechanisms that govern their transport in the environment. However, this information could be used to develop strategies for the biomonitoring of the pathogen and suggesting measures to limit their dissemination in the environment in equatorial regions of Cameroon. Considering the growing number of piggeries along some river courses in Yaoundé, and also the direct release of their effluents into the aquatic system, our research seek to investigate if these definitive hosts of *B.* coli did not enhance the spread of the resistant forms into the aquatic system. This research also seek to study the influence of ecological factors on the occurrence of *Balantidium* cysts in tropical aquatic ecosystems which are directly exploited by the population for domestic chores, by riverside traders to clean their goods, clean the slaughtered pigs and by farmers for irrigation of their crops. Due to the direct contact between the aquatic system and the riverside inhabitants, our diagnosis will evaluate the sanitary risks linked to the transmission of these entero-pathogens in tropical aquatic ecosystem that is predominantly used in urban agriculture in Yaounde.

MATERIALS AND METHODS

Sampling locations and site information

Our study site which is located in Yaoundé, the political capital of Cameroon lies between latitude 3° 51 and 3° 52 north and between longitude 11° 31 and 11° 33 east. The Ewoue stream receives a direct piggery effluent (pig market at Mvog Ada) as presented in Figure 1. It is also characterized by the presence of domestic waste such as polyethylene bottles and domestic garbage. Sampling points have been chosen at the source (denoted E_1), the piggery effluent (E_3), upstream and downstream of this effluent (E_2 and E_4), and the outlet ($E5$) where the watercourse enters the mainstream in the municipality of Yaoundé. This research took place from November 2011 to Mid March 2012 which is the Long Dry Season (LDS) and from Mid March to May 2012 which is the short dry season (SDS) in the equatorial regions of sub-Saharan Africa.

Physico-chemical and hydrological parameters

The samples were collected following a monthly frequency, and the data was assessed on seasonal basis. Hydrological parameters which were taken into account were the flow rate and the longitudinal profile of the river. The flow rate gives an estimation of the transport of the cystic load within the water column. The flow rate was assessed by determining the time taken by the front of blue methylene to travel a given distance, and is calculated after assessment of the wet section by applying water following formula:

($Q = v \times s$)

Q = Flow rate in m^3/s, v = velocity in m/s, s = wetted section in m^2

Physicochemical analyses were carried in the field and in the laboratory of Hydrobiology and Environment of the University of Yaounde 1, following the procedure of Rodier et al. (2009) and APHA (2009) recommendations. Samples were collected in each sampling point on monthly basis for physico-chemical analysis. Temperature was measured on the field using a mercury thermometer. The potential of hydrogen (pH), conductivity and total dissolved solids were measured *in situ*, using a pH-meter model HACH to the nearest 0.1 CU and a TDS-conductivity meter HACH to the nearest 0.1, respectively. Dissolved carbon dioxide was fixed in the field and then analyzed in the laboratory. In the laboratory, water color and suspended solids were measured using a spectrophotometer DR/2800, while alkalinity and calcium hardness concentration were determined by titration method with the application of the appropriate indicators as presented by Ajeagah (2013). Biochemical oxygen demand (BOD_5) was estimated using a BOD-meter at 20°C in dark conditions.

Biological parameters

On the site, the choice of the sampling place was conducted by an accumulation of organic matter and the presence of the colonising herbs. The stream was gently agitated so as to put the particles into suspension. The double plugging 1000 ml polyethylene bottles were used for sampling, after previous rinsing with demineralised water and water from the environment. In the laboratory, the samples were allowed to settle for 24 - 48 h and the pellet, whose specific volume is noted, was collected. The observation of cysts was made directly to the inverted Olympus CK2 microscope at a magnification of 400 and the 1000x after application of the physical technique and the two-phase methods of concentration. These different research procedures are complementary methods that enable an optimal isolation and identification of *Balantidium* cysts in water. Their specific concentration and application procedure is presented herein.

Physical methods

One of the techniques that were used in this assessment was the concentration with distilled water. After homogenization of the pellet, 5 ml of the sample was collected and placed in a test tube. 1 ml of formaldehyde (fixative) and 5 ml of distilled water were successively added to the sample. To facilitate the sedimentation of the cysts, the mixture was centrifuged at 500 turns / min for 5 minutes. After adding two drops of Lugol, a drop of sample was removed, placed on a microscope slide and covered with a cover slip for identification and enumeration of cysts.

The concentration of zinc sulphate was the second technique applied. It helps in the concentration and flotation of the pathogens to be analysed. It was carried out following the steps described above, except that 5 ml of distilled water used were replaced with 2 ml of distilled water and 3 ml of zinc sulphate.

Diphasic methods

According to the Ritchie method (Mora, 2010), 3 ml of the pellet were placed in a test tube. 7 ml of 10% formalin and then a minute later 3 ml of ether were added. The mixture was mixed manually and then centrifuged at 500 turns/min for three minutes. Four layers were observed in the test tube after homegenisation. Then the fat cap (debris) was removed with a stick, and the supernatant was made by inverting the tube with a quick motion. Finally, the pellet was mixed with two or three drops of Lugol which was used for the identification and enumeration of cyst after mounting between the slide and cover slide. The concentration technique of Telemann-RIVAS (Lacoste, 2009) was performed according to the same protocol as the formalin-ether concentration. The only differences are seen in the dose and reagents used. So we used 5 ml of acetic acid at 5% and 5 ml of ether. All these methods of concentration have yielded the same results count and the cysts were identified on the basis of their morphology. The number of cysts contained in 1 L of sample was obtained by the formula proposed by Ajeagah et al. (2010). This formula states that the number of cysts enumerated(x) is given by the formula:

$$x = \frac{y.Vx}{Vy}$$

Where, Vx = Pellet volume of 1 L of sample; Vy = Pellet volume considered in the identification; y = Number of cysts counted in Vy

Statistical analysis of the abiotic and biotic variables

Canonical analysis of correspondence was carried out to measure the level dependence between the hydrological, physico-chemical, biological variables and the sampling points assessed in our study. It was carried out with the help of the PAST program (Hammer et al., 2001). The ecological variables measured were also tested with the ANOVA test associated to the student t test, using the SPSS Program, version 17.0 and the results were appreciated at 1 and 5% security level. Pearson rang correlation (r) was applied to measure the level of relationship between the biotic and the abiotic parameters. The Bray Curtis index was applied to measure the level of affinity between the different sampling stations that have been considered in our investigations (Nébout et al., 2010).

RESULTS

Spatio-temporal variations of physical, chemical and hydrological parameters

Hydrological and physicochemical characteristics of rivers are presented in Table 1 which shows the seasonal values of each parameter at each sampling site. Readings of the water velocity shows a sharp decrease

Table 1. Physicochemical and hydrological characteristics of the Ewoue water system during the sampling period.

Station	Seasons	Temperature (°C)	pH	Conductivity (*u*S /cm)	CO_2 (mg/L)	O_2 (mg/L)	Alc (mg/L)	Ca (mg/L)	Oxyd $KMnO_4$ (mg/L)	TDS mg/L	Turbidity NTU	Colour Pt.Co (TCU)	SS (mg/L)	PO_4 (mg/L)	NO_3- (mg/L)	BOD_5 mg/L O2	Speed (m/s)	Flowrate (m^3/s)
Ewoué 1	LDS	25.25	4.88	230.00	5.28	43.57	5.00	4.00	0.70	103.20	1.40	58.00	14.00	0.64	0.08	25.00	0.50	0.00
	SRS	25.00	6.41	299.67	19.36	4.04	12.00	16.00	1.60	152.00	0.83	13.67	3.00	22.11	0.07	76.67	1.37	0.00
	Average	25.13	5.65	264.83	12.32	23.80	8.50	10.00	1.15	127.60	1.12	35.83	8.50	11.38	0.07	50.83	0.93	0.00
	St.D	0.18	1.08	49.26	9.96	27.95	4.95	8.49	0.64	34.51	0.40	31.35	7.78	15.18	0.00	36.53	0.61	0.00
Ewoué 2	LDS	26.67	7.30	327.65	13.20	10.69	151.33	24.67	3.50	260.00	12.00	372.00	75.00	2.29	0.01	50.00	0.11	0.13
	SRS	25.83	7.12	488.33	8.21	21.42	194.00	29.33	9.12	247.00	27.33	284.67	30.67	3.57	0.03	106.67	0.09	0.14
	Average	26.25	7.21	407.99	10.71	16.06	172.67	27.00	6.31	253.50	19.67	328.33	52.83	2.93	0.02	78.33	0.10	0.14
	St.D	0.59	0.13	113.62	3.53	7.59	30.17	3.30	3.97	9.19	10.84	61.75	31.35	0.91	0.02	40.07	0.02	0.01
Ewoué 3	LDS	30.67	7.28	342.35	116.16	13.93	175.33	82.00	4.05	270.00	20.00	1056.00	19.60	6.65	0.05	90.00	0.25	0.36
	SRS	25.50	7.13	558.00	11.15	20.27	211.33	24.67	8.70	277.00	52.00	671.00	77.33	8.75	0.12	146.67	0.24	0.28
	Average	28.08	7.20	450.18	63.65	17.10	193.33	53.33	6.38	273.50	36.00	863.50	48.47	7.70	0.08	118.33	0.25	0.32
	St.D	3.65	0.10	152.49	74.26	4.48	25.46	40.54	3.29	4.95	22.63	272.24	40.82	1.48	0.05	40.07	0.01	0.06
Ewoué 4	LDS	26.67	7.18	350.80	8.80	4.40	234.00	79.33	4.55	290.00	48.00	638.00	214.00	1.00	0.02	95.00	0.23	0.16
	SRS	24.67	7.16	545.33	5.87	11.32	231.33	102.00	6.92	272.00	27.33	379.33	35.67	1.95	0.01	153.33	0.64	0.42
	Average	25.67	7.17	448.07	7.33	7.86	232.67	90.67	5.74	281.00	37.67	508.67	124.83	1.47	0.02	124.17	0.43	0.29
	St.D	1.41	0.01	137.56	2.07	4.89	1.89	16.03	1.68	12.73	14.61	182.90	126.10	0.68	0.00	41.25	0.29	0.19
Ewoué 5	LDS	26.25	7.35	560.00	7.04	40.30	246.00	16.00	3.00	275.00	10.00	236.50	16.00	28.65	0.02	55.00	0.56	0.11
	SRS	24.00	7.18	556.67	6.45	7.14	236.00	34.67	5.80	286.00	26.67	272.67	22.33	1.73	0.00	123.33	0.87	0.20
	Average	25.13	7.26	558.33	6.75	23.72	241.00	25.33	4.40	280.50	18.33	254.58	19.17	15.19	0.01	89.17	0.71	0.16
	St.D	1.59	0.12	2.36	0.42	23.44	7.07	13.20	1.98	7.78	11.79	25.57	4.48	19.03	0.01	48.32	0.22	0.07

of this variable at the source where it is maximum (1.367 m/s in the short rainy season (SRS) and 1.01 m/s in long dry season (LDS)) to E_2 station where it is minimal (0.09 m/s in LDS and 0.11 in SRS). It then gradually increases from E_2 to the outlet. The general observation is that, the values obtained by SRS are higher than those obtained by LDS.

Based on the near-zero values for E_1 (1.2 and 1.8. 10^{-4} m3 / s), the flow rate of Ewoué gradually increases until it reaches it's maximum value at E_4 for SRS (0.36 m3 / s) and E_3 for the LDS (0.42 m^3/s). This rate then decreases to E_5. Except in the effluent (E_3), the water temperatures vary little around the average temperature obtained during the sampling period (25.99 ± 1.99°C) as well as around the average air temperature as recently published by the Regional Meteorological Centre (24.55 ± 2.39°C). This station shows an exception during the LDS with a temperature of 30.67°C. The values of turbidity of the Ewoué are low at the source sampling point (0.83 FTU in SRS and 1.4 FTU in LDS) and higher than or equal to 10 FTU in other stations. Overall, the values obtained by LDS are lower than those obtained in SRS. The colour of the water is more pronounced in LDS (58 to 1056 U Pt-Co) than in SRS (13.67 to 671 U. Pt-Co). In terms of space, except for the source waters of the Ewoue stream are highly coloured and the maximum values are recorded in E_3.

The maximum levels of suspended solids and total dissolved solids were recorded at E_3 for SRS (respectively 77.33 and 266 mg/l of sample) and

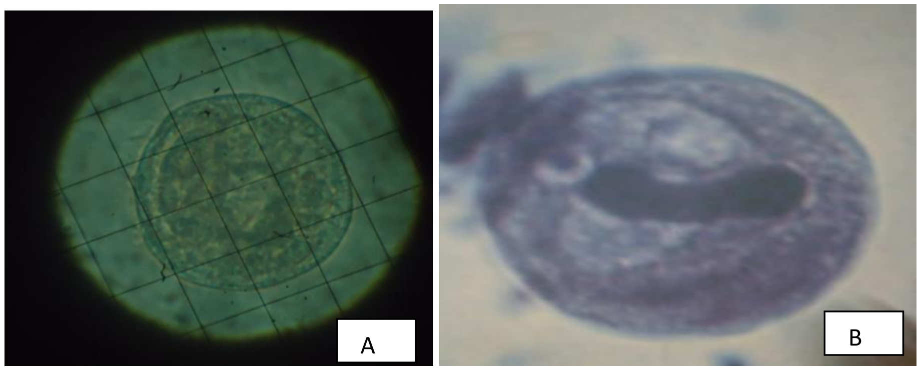

Figure 2. Forms of *Balantidium coli* cysts observed. Round cyst 30 µm (A) and 40 µ oval cyst (B).

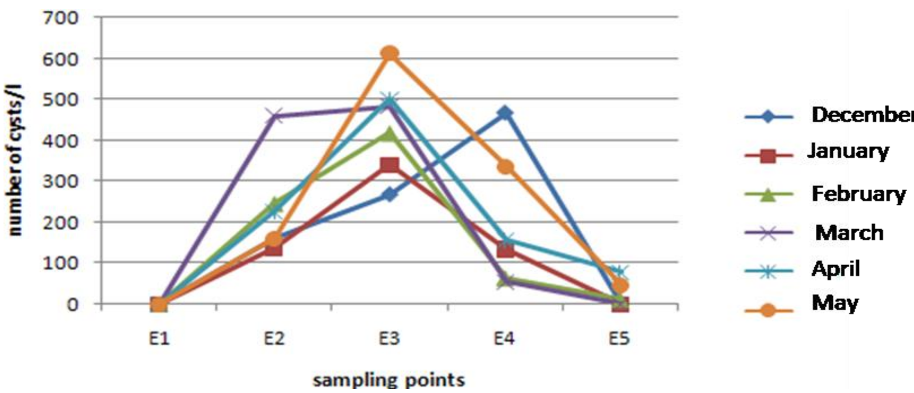

Figure 3. Monthly variation of the densities of cysts in the different study items.

E_4 in the LDS (respectively 214 and 290 mg/l of sample). As for minimum levels, they appear at the source (respectively 3 and 103.2 mg/l of sample). Values of pH and alkalinity rose gradually from the source to the outlet. The minimum values are 4.88 and 6.41 CU for pH and 5 and 12 mg/l of sample for alkalinity while the highest values recorded are 7.18 and 7.35 for pH and 236 and 246 mg/l of sample for alkalinity. On the seasonal plan, these two parameters move in opposite directions. The maximum values are all obtained during the LDS for pH and during the SRS for alkalinity.

The concentrations of dissolved CO_2, both in LDS and SRS, remain below 20 mg/l except at the effluent where it reaches 116.16 mg / l in LDS. As for dissolved oxygen, its curves show very low levels throughout (<25%). Values of 43.57 and 40.3% are exceptionally obtained at E_1 and E_5 during the LDS. The values of oxidability and BOD_5, are higher in SRS than in LDS. These values are generally very high and fluctuate between 0.70 mg/l O_2 (E_1) and 9.12 mg/l O_2 (E_3) for oxydability and between 23 mg O_2/l (E_1) and 153.33 mg O_2/l (E_4) for BOD_5. The electrical conductivity is significantly higher in the rainy season than in the dry season. The highest value was observed at the outlet (560 µS/cm) and the lowest at source (230 µS/cm). The average nitrate ranged between 0.003 and 0.12 mg/l of sample. The maximum and minimum levels are obtained during the SRS in points E_3 and E_5. As for orthophosphate, the extreme concentrations observed in E_1 and E_5 are respectively 0.64 and 28.65 mg/l during the LDS and 22.11 and 1.73 mg/l in the SRS. The results of the study reveal the concentrations of calcium hardness between 4 and 102 mg/l of sample. The maximum value was observed in SRS and the minimum in LDS.

Spatio-temporal variation of biological parameters

Balantidium coli cysts sampled in the river Ewoue during the study period have sizes between 30 and 75 µ and are in two forms. The round shape and the oval shape (Figure 2A and B). A total of 5356 cysts of *B. coli* are enumerated in our study. The monthly densities vary between 0 and 613 cysts/l depending on the sampling point and the hydro-ecological properties of the station considered. Overall, the monthly number of cysts counted in E_3 (269-613) is still the most important. The spring water has no cyst and the density of cysts does not exceed 78 cysts/l at the outlet (Figure 3). During the

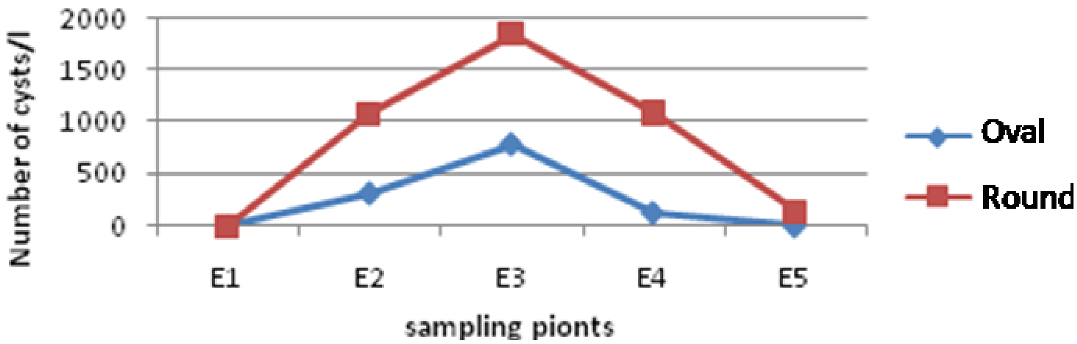

Figure 4. Spatial variation of the densities of different types of cysts.

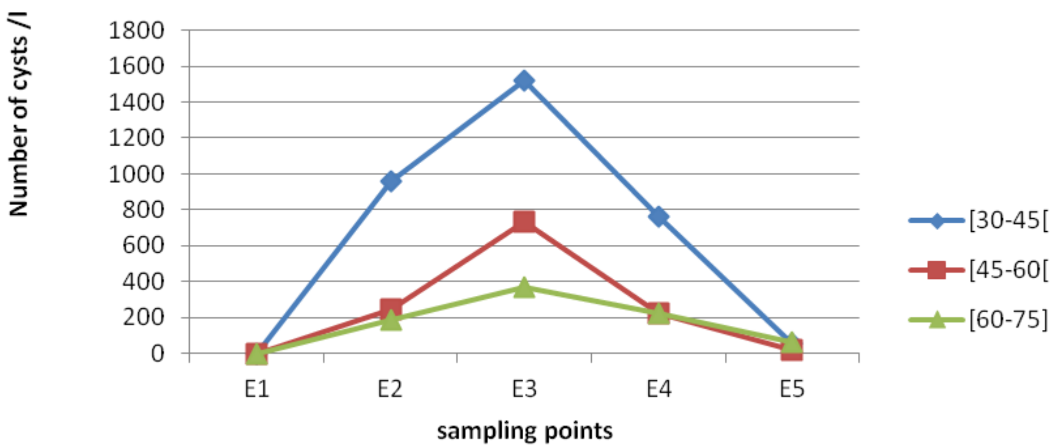

Figure 5. Spatial variation of the densities of different size classes of cysts.

study period, the spatial distribution of different types of cysts showed a clear dominance of round cysts over the oval forms. The round shapes cysts, as well as the oval have their maximum densities at E_3 (1835 and 787 cysts, respectively for oval and round forms) (Figure 4). Three classes of cysts are distinguished in our assessment. The class of smalls cysts is 30-45 µm, that of mediums size cysts is 45-60 µm and, that of larges cysts are 60-75 µm. These curves show higher densities of smalls cysts (958 cysts/l at E_2, 1524 cysts/l at E_3 and 763 cysts/l at E_4) followed by medium-sized cysts (244 cysts/l at E_2, 730 cysts/l at E_3 and 525 cysts/l at E_4) and larger sizes cysts that vary between 0 cyst/l and 367 cysts/l (Figure 5).

Based on 0 cyst/l at E_1, the curves of average densities of the seasons cysts describe parabolas whose peaks are at E_3 (means values = 342 cysts/L during the LDS and 531 cysts/L during the SRS). Overall, the average density values obtained during the SRS are more important than those obtained during the LDS (Figure 6). On the seasonal level, the observed shapes are more in SRS than in LDS. However, even in LDS, and regardless of the sampling station, the number of round cysts (0 to 360 cysts/l) is higher than the oval cysts (0-171 cysts/l SRS). Both spatial and seasonal average densities of small cysts undermined other densities. The highest concentrations of the cysts in the aquatic medium are obtained at the effluent of piggery that is linked to a main market.

Changes in abiotic parameters around biotic parameters

The analysis of canonical correspondence reveals the specificities of each station. E_1 is characterized by the absence of cysts, the acid pH, BOD_5 lower than elsewhere. The sampling points E_2 and E_4 present high values of calcium hardness, turbidity and *B. coli* cyst. E_3 has a high abundance of cysts and maximum water coloration. As for E_5, it is characterized by a maximum value of electrical conductivity and alkalinity (Figure 7). The ANOVA test associated with Student "t" test reveals that the colour and alkalinity were significantly lower in the E1 station from other stations (p = 0.017 and 0.027

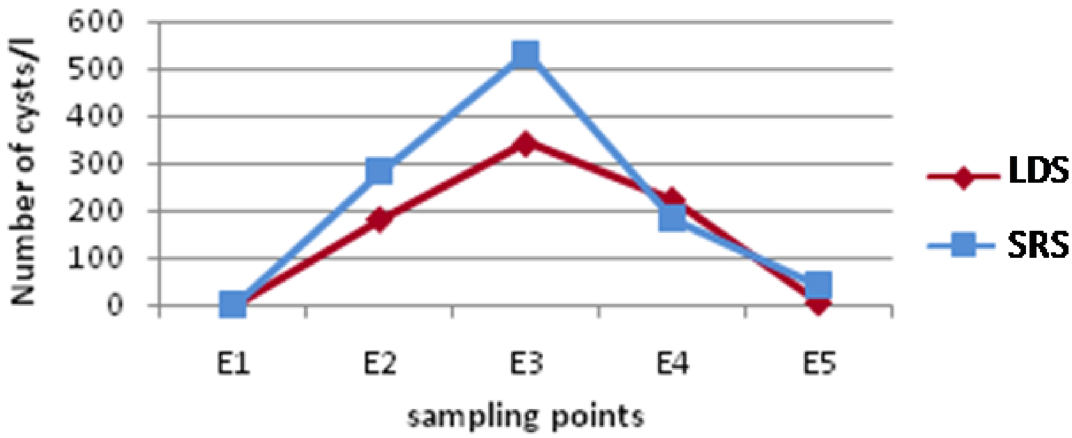

Figure 6. Seasonal variation of cysts in various research stations.

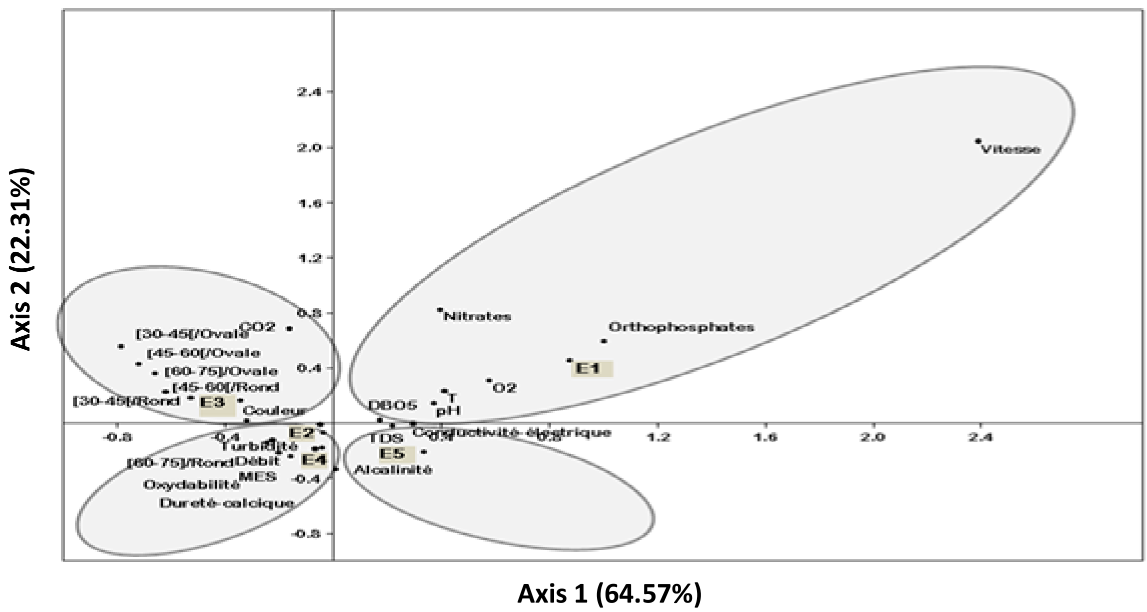

Figure 7. Canonical correspondence analysis (CCA) of sampling stations in the variables measured.

for the colour alkalinity between E_1 and E_2). The density of cysts are significantly higher in the E_2, E_3 and E_4 stations. The differences are more precisely between E_1-E_2, E_1-E_3, E_1-E_4, E_5-E_4, E_5-E_3 and E_5-E_2 when you consider all the cysts. The rounds cysts are identified between E_4-E_5, E_1-E_3, E_2-E_1, E_3-E_5. The small cysts are enumerated between E_1-E_2, E_2 - E_3, E_4-E_5, E_1-E_3, E_1-E_4. The KHI-2 tests performed proof that there is a strong tendency to identify round and small cysts ($p = 2.7557. 10^{-8}$), and medium-sized oval cysts ($p = 2.7557. 10^{-8}$) at all the stations considered. They also reveal that the round and small cysts are predominant in all stations of the ecosystem. With a risk of 1%, a significant correlation between flow and calcium hardness ($r = 0.794$, $p = 0.006$) was noted. For a risk of 5%, the flow moves in the same direction as the alkalinity ($r = 0.645$, $p = 0.044$), the oxidability ($r = 0.639$, $p = 0.047$), colour ($r = 0.710$, $p = 0.021$) and BOD5 ($r = 0.751$, $p = .0.0035$). CO_2 dissolved in the river moving in the same direction as the temperature ($r = 0.886$, $p = 0.001$). It is the same for ammonium and turbidity ($r = 0.770$, $p = 0.009$), and colour and temperature ($r = 0.767$, $p = 0.010$), pH and alkalinity ($r = 0.827$, $p = 0.003$), alkalinity and electrical conductivity ($r = 0.791$, $p = 0.006$), pH and TDS ($r = 0.834$, $p = 0.003$), alkalinity and TDS ($r = 0.750$, $P = 0.013$).

The significant correlations found between the hydrological, physicochemical parameters and the

Table 2. Significant correlations obtained between the physical, chemical and hydrological and shapes, sizes and densities of cysts.

Physical, chemical and hydrological variables	Shapes of cysts		Sizes of cysts			Densities
	Round	Oval	[30-45[	[45-60[	[60-75[	
Oxydability	0.879*	0.778**	0.747*	0.851**	0.746*	0.853**
TDS	0.634*	0.362	0.646*	0.445	0.432	0.598
Turbidity	0.780**	0.679*	0.609	0.696*	0.889**	0.752*
Color	0.769**	0.314	0.842**	0.595	0.466	0.762*
Flow	0.671*	0.192	0.673*	0.549	0.242	0.607

* = Significant correlation at 5% **= Significant correlation at 1%.

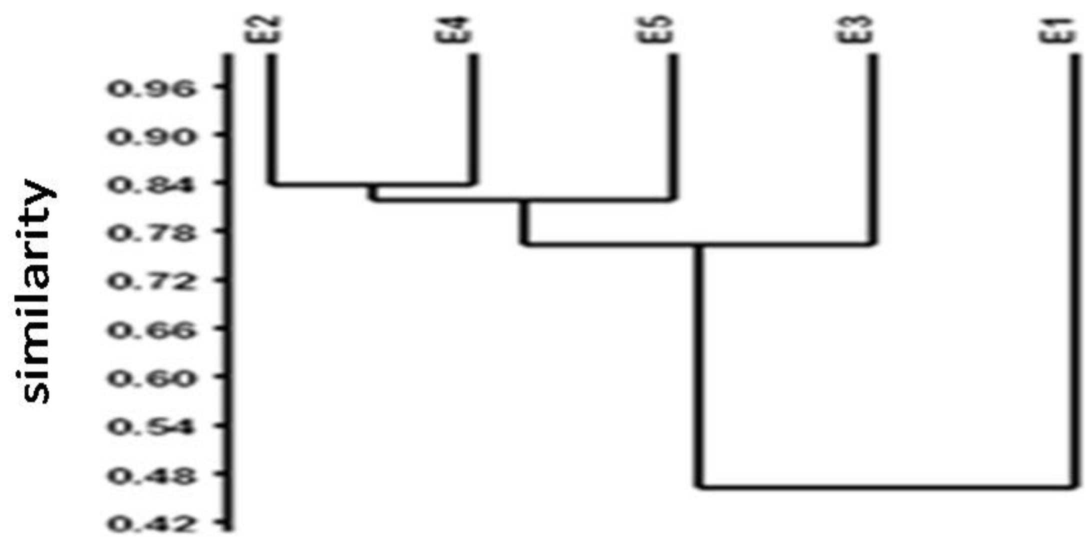

Figure 8. Dendrogram showing the similarities between the stations based on the parameters measured.

structural attributes of the environmental forms are summarized in Table 2.

The similarities between the sampling stations based on the hydrological, physico-chemical and biological variables assessed are presented in a dendrogram (Figure 8). It presents an 84% similarity between stations E_2 and E_4 which share the same branch and directly receive the piggery effluent. The ecological niches consisting of E_2, E_4 and E_5 has 76% similarity with E_3 and only 45% similarity with E_1 which is the unpolluted sampling point at the source(Figure 8).

DISCUSSION

A non-significant spatial-temporal differences between the hydrological, the biological (Figure 6) and the physico-chemical parameter assessed in this stream are due to the low variation between the LDS and the SRS in tropical Africa. According Suchel (1972), the meteorological characteristics of Yaoundé varies gradually over time. However, some variables such as organic matter, turbidity, cysts density are more important in the SRS than in the LDS. This is due to the accumulation in the bottom sediment organic and inorganic matters which are released into the water column under the action of rainwater (Ajeagah et al., 2010).

The irregularity of speed from upstream to downstream (Table 1) is due to different degrees of inclination of the slopes. This rate is lower at E_2, because the slope at this point is lower (Table 1). Hebert and Légaré (2000) noted that when the slope of a river decreases, the water flow rate decrease. This slows the runoff of bio-contaminants such as the cysts of *B. coli* in the aquatic ecosystem, thereby increasing the possibilities of contacts with the riverside population who exploit this hydrosystem for their domestic purposes.

The gradual increase in the flow from the source to E_4 (Table 1) is influenced by the input of the different tributaries along the stream course. Leveque (2001) suggested that the wet section of a watercourse increases when it receives small tributaries. These stations receive essentially latrine effluent and farm bio-contaminants. This will justify the increasing levels of cysts from the source to the piggery effluent. The abundance of *B. coli* cysts should be most important at E_4 than at E_3. Contrary results could be explained by the presence in the bed of the stream of solid substrates such as, hundreds of polyethylene bottles that have retained cysts and acts as a mechanical filter of the water. Between E_4 and E_5, reducing the flow could be justified by infiltration along the way, through activities such as irrigation of fields that require the retention of water. This water retention in the fields could induce sedimentation of cysts at this level thus explaining the decrease in their content at the outlet.

The turbidity of the analysed waters was significantly correlated with the content of organic matter due to contamination by poorly soluble organic waste mainly from households and farms. In this regard, Arfi et al. (2003) argues that most of the water turbidity is due to the presence of suspended particles in water. Positive correlations between suspended solids, turbidity and the density of cysts are due to the fact that SS ensure their dissemination in water. In the water, oocysts and cysts of parasites are usually associated with organic matter in suspension (Medema et al., 1998). This association is caused by electrostatic interactions such as those of the hydrogen bonding, Lifshitz-Van der Waals and Lewis acid-base (Dai and Boll, 2003; De Jonge et al., 2004). These results corroborate those obtained by Ajeagah et al. (2007, 2010) which proof that there is a positive correlation between turbidity and the different forms of resistance to enteric pathogens which have been isolated in two streams of Yaoundé that are subjected to organic pollution.

The pH values recorded during the study period indicate that Ewoue waters are acidic to neutral (Table 1). These measures are similar to those obtained by Ebang (2002) on the same stream. The acidity of the water source is due to the ferralitic acidic nature of the soil in Yaoundé.

Leynaud and Verrel (1980) concluded that the effect of a natural water pH is closely related to the soil. It's evolution towards neutrality in the other stations could be explained by a contamination of waterways by varied domestic and industrial effluents. High levels of orthophosphate as presented in Table 1 confirm it. The strong correlation between alkalinity and pH ($r^2 = 0.827$, $p = 0.003$) also shows that the basic pattern of this water is mainly due to alkaline ions in solution. These bind electively on cystic membranes inducing them to break up in the medium (Crevieu-Gabriel and Naciri, 2001). This process of excystation could also be the cause of low cysts count at the outlet of this urban water system.

The water temperatures of the different stations (Table 1) are similar to those observed in the work of Ebang (2004) on the same stream and close to the average temperature of air recorded by the regional meteorological centre between January 1996 and June 2010 (24.55 ± 2.39°C). Liechti et al. (2004) shows that the change in the temperature of flowing water is in line with the air temperature. Only E_3 station presented a slight difference in LDS (30.67°C). At the Mvog Ada market, the creeks undergo discontinuous thermal pollution. Cleaners of pigs, chickens and goats release hot water into the stream as they clean the slaughtered animals. This creates a thermal gradient that induces the activation of the enzymes needed for excystation.

The distribution of cysts in the river (Table 1 and Figure 7) could also be explained by the direct action of man. The fact that some parts of the source (E1) are cemented, limit seepage and contamination of ground water through infiltration (Besassier et al., 2006). Even when infiltration takes place, the microorganisms are retained by the soil horizons (Nola et al., 2003, 2004). The retracted position of the outlet would protect human activities (farming and farm waste disposal, wastewater disposal waterfront homes and faeces from latrines) (Figure 8). The highest concentrations of the cysts of *B. coli* enumerated midstream are mostly due to the piggery effluent. Pork is the main source of contamination (Visvesvara and Schuster, 2004). According Bouhoum (1996), the number and variety of pathogens found in wastewater is related to the level of infestation of human or animal population present. Ajeagah et al. (2007) shows that the drainage system of Mfoundi streams of Yaoundé are subject to a high faecal pollution and constitute an important input of concomitant infectious agents.

Round cysts are higher than ovals cysts as indicated on Figures 4 and 5. Similarly, small cysts are more abundant than larger ones. Studies by Wang et al. (2011) have shown the existence of two genotypes of *B. coli* in the ecosystem. There are genotype A that produces large trophozoites, low mobility and are fewer in faeces and culture media unlike genotype B trophozoites which are smaller and more numerous in the samples examined. This could justify the frequency of different cysts sizes in the aquatic environment analyzed.

Conclusion

The results presented in our study reveal that the aquatic system is completely devoid of *B. coli* cysts at the source and presents a maximum density at the piggery effluent located at the midstream. Although at the outlet, Ewoue tends to regulate its ecological balance, a significant cystic load is discharged into the Mfoundi mainstream of Yaoundé which is exploited for the production of drinking water and household chores in Yaoundé and its environs. There is a predominance of round and also small sized (30-45 µm) cysts in the river system. Dissemination of cysts in water increases with turbidity and suspended solids that adhere to the resistant forms of the pathogens by the mechanism of adsorption. The population is exposed to contamination with *B. coli* cyst by exploiting the aquatic medium for cleaning the pigs that are sold in the urban market, in urban agriculture and by children who use the water for recreational purposes. The procurement of pipe borne water in this pig market could reduce the usage of the polluted water of Ewoue in cleaning the slaughtered animals and thereby reduce the spread of intestinal diseases in the Yaounde municipality.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Land degradation assessment of agrarian soils in Ebonyin State for sustainable production

Ande O. T[1*]. and Senjobi B. A[2].

[1]Institute of Agriculture Research and Training, OAU, Moor Plantation, Ibadan, Oyo State, Nigeria.
[2]University of Agriculture, Abeokuta, Ogun State, Nigeria.

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The experiment was conducted to assess land degradation in agrarian soils of Ebonyin State. Ten farmers' fields were selected based on land use and landscape positions across the three zones in the state. Physical and socio- economic information were obtained from the farmers and extension agents. Surface and sub-surface soil samples were collected for physical and chemical analysis. The soils at the upland were clay loam and at lower slope to valley bottom were clay loam to clay in texture. The upland and low land soils were strongly acidic (4.1) to moderately acidic (5.5) with low P (3.36 - 5 mg/kg). The soils also were low in cation exchange capacity (CEC) (1.36 - 2.68). Soil degradation indicators include crop conditions, poor yield, low fertility level, shallow soil depth (50 cm), high gravel content (> 35%), compaction of exposed sub soils and soil erosion. The factors and causes of soil degradation include improper tillage system, continuous cultivation, nutrient mining due to many crops per heap under mixed cropping and improper crop combination. Conservation agriculture with minimum tillage and green manure are recommended for sustainable production while combination of organic and in-organic fertilizer is recommended to improve soil quality.

Key words: Land use, soil degradation indicators, tillage systems, cropping systems and poor yield.

INTRODUCTION

Land degradation will remain an important global issue for the 21st century because of its adverse impact in agronomic productivity, environment, food security and quality of life. The existing technology have invited host of problems of production declining land quality and degradation of soils of the areas. The productivity of some soil had decline by 50% due to erosion and desertification (Pagiola, 2002). Yield reduction in Africa due to past soil erosion ranges from 2 - 4%. In Asia, annual loss in productivity is estimated at 36 milliontonnes of cereals. Land degradation is a decline in soil qualities, which can be considered in term of issue of actual or potential productivity or utility as a result of natural or autotrophic factors (Beinroth et al., 1994).

Land clearing, agricultural depletion of soil available nutrients status/immobilization, irrigation, pollution, accelerated erosion by wind or rain acidification or alkalinezation, salinization destruction of soil structures through the loss of soil organic matter are prevalent causes and processes that easily result to land degradation in Nigeria. Soil compaction hard crusting is a worldwide problem, especially with adoption of mechanized agriculture.

*Corresponding author. E-mail: funmiande@yahoo.com.

It has caused yield reduction of 25 - 50% in some regions of Europe (Erickson, 1999) and between 40 - 90% in West Africa (Lal, 1994). Nutrient depletion as a form of land degradation has severe Economic impact at the global scale, especially in sub-Sahara African. Annual depletion rate of soil fertility was estimated as 25 kgN/ha, 3 kgP/ha and 15 kgK/ha (Nyathi et al., 2003).

Soil fertility decline refers to deterioration in soil, the physical and chemical, biological properties, by reduction in soil organic status leads to decline in soil biological activities. Degradation in soil physical properties structure, aeration, water holding capacity caused by reduced organic matter, adverse change in soil nutrient status including reduction in availability of major nutrients (NPK), initiation of the micro nutrient deficiencies and development of nutrient imbalance.

Land degradation is a reduction or loss of biological economic productivities of crops and or range pasture, forest wood land resulting from land use or from processes of soil erosion caused by wind results in and results in deterioration of the physical, chemical and biological economic properties of soil and long terms loss of natural vegetation (Evenson and Gollin, 2003).

There are certain indicator of degradation that can be assessed in different land use pattern (Worden et al., 2003). indicators include change in hydrologic regimes in a forest by the use of epiphyte such as mosses and bryophytes; change in soil reaction (acidity and alkalinity) due to the salinization or acidification; change in nutrient status of the soil such as fertile or poor soil; crop yield data (Table 1b) and plant conditions during the growth stage.

MATERIALS AND METHODS

Field study was conducted on selected farmers' field based on their cropping system and terrain characteristic. The selected sites were characterized and relevant data on soils, its climatic, crop production problems and history, landscape and yield were collected. Soil samples were collected in three replicates from surface and subsurface from plots of 5 × 5 m marked on the farmers' field. The samples were processed for physical and chemical analysis.

The results were interpreted to determine factors of land degradation and appropriate intervention for sustainable land use; the physical and chemical analysis carried out on the soil samples. Particle size analysis was done using Bouyocos (1999) method, bulk density was determined by core method. Cations were extracted by using NH_4OAC (Ammonium Acetate) Ca and Mg were read with atomic absorption spectrophotometer Na^+ and K^+ by flame photometer. Walkley black method was used for organic carbon determination.

Phosphorus was determined using Bray1 method. The cation exchange capacity (CEC) was calculated by sum of bases. Nitrogen value was derived from organic carbon content.

Land degradation assessment by direct approach

Random on each farm land use were placed in degradation classes by soil characteristics with the land indicators (Tables 4 and 5). Classification was done following the approach of FAO (1979) and Snakin et al (1996). The estimation of degree of degradation was based on physical, chemical and biological properties of soil and parameters of land use types at location were studied. The soils were ranked based on degree of degradation.

RESULTS AND DISCUSSION

Landscape, land use and cropping systems

The study areas is characterised by undulating to rolling landscape. Sites were located at different positions on the landscape from upland to valley bottoms. The upland soils were used for cassava, maize, potato, yam and vegetables activation. While the valley bottom soil were reserved for rice cultivation (Table 1).

Mixed cropping is the usual practice at the upland while mono-cropping of rice is the practice at the lower slope. The upland soils are usually cultivated intensively for four years before being left for fallow while rice is usually cultivated continuously. Agro-forestry is practiced close to homestead.

The average soil depth on the farm was about 50 cm with laterite in the sub-soil. The rainfall ranges between 1000 to 12000 mm per annum.

Physical and chemical properties

The data of physical and chemical analysis of the surface and sub surface soils were indicated that (Tables 2, 3) the soils were having high clay content which ranged between 31.2 to 56.2% in both upland and valley bottom soils with an average soil depth about 50 cm.

The chemical analysis of soil sample showed that the soils were strong acidic (pH 4.1) to moderate acidic pH 5.5 with low P content (3.36-5.44 mg/kg soil). CEC ranged between 1.25-2.68 cmol/kg soil which could have resulted in poor yield of the crops in the area. Higher CEC 6.252- 8.92 cmol/kg was recorded form surface to sub surface in agro forest areas.

The soil fertility is usually maintained by shifting cultivation and application of fertilizer. However soils under fallow period of 2 to 3 years were also found to be in low fertility. This indicates that the fallow period is too short to build up soil fertility (Table 2).

Interaction with farmers showed that one bag fertilizer (NPK 15:15:15) per acre was the usual practice. This is below the crop requirement on low fertility soils for profitable production. The data on land degradation indicators (Table 4) shows that 60% of the studied area was highly degraded. 90% of the area was highly deficient in N, 50% soils were moderate to highly deficient in P, 60% was moderately degraded in K. 60% of the area was found to be slightly degraded in organic matter while 20% of the soils was very low in organic matter content. Bulk density of the soils ranged between 1.10 to1.60 which resulted to 60% highly degraded and 40% moderately degraded.

Table 1. On-farm land degradation assessment in Ebonyin State.

Landscape position	Land use/Cropping system	Degradation indicator	Management levels
Valley bottom	Rice (sole)	Small panicles, stunted growth, ferric solution.	Medium
Upland	(Mono-cropping) Yam /cassava / okra	Thin stems, Leaf chlorosis, stunted growth, small tubers gravel soils	Medium
Upland	Agro-forestry	Soil colour	Low
Upland	Maize/yam/cassava	Sub surface Soil compaction, gravel soil	Medium
Upland	Fallow	Grasses	Medium
Lowland/valley bottom	Rice (Mono-cropping)	Small panicles, stunted growth	Medium
Lowland/valley bottom	Yam/cassava /Okra rice sole.	Ferric solution, iron toxicity	Medium
Upland	Yam, cassava, potato, okra	Thin stems, small tubers, low yield, gravel/plinthile	Medium
Fallow	-	Grasses	Medium
Lowland	Maize, cassava, potato	Low yield	Medium

Table 1b. Average crop yield in Ebonyin State

Location	Land use	Cropping system	Yield (t/ha)
1	Rice	Sole	1.5
2	Cassava	Mixed cropping	5.0
3	Cassava	Mixed cropping	5.0
4	Maize	Mixed cropping	1.6
5	Rice	Sole	1.5
6	Rice	Sole	1.5
7	maize	Mixed cropping	1.8

Land degradation indicators: Soil fertility, cropping system and tillage methods

The continuous production on soils for over two decades without sufficient fertilization and manuring has resulted in low soil fertility status of the soils. Shallow soil depth and tillage practices have also contributed to soil degradation. The usual traditional method of tillage involves making large heaps by scraping surface soils to about 50 cm depth. This practice has resulted to exposure of sub soils, formation of large furrows aiding erosion activities and sub surface hard crusting. Ridges were made along in the area creating channels for soil erosion. This has been attributed to accelerated soil erosion with consequent loss of top soils in the area. The over utilization of lands under mixed cropping was also observed as one of land degrading factors. Similar observation was made by Michael and Niamh (2000). Improper root crops combination (such as cassava/yam/potato mixtures) with 5 to 10 cassava plants on a heap and other crops such as maize and okro is the usual practice. Hence unhealthy competition for scarce soil nutrient is inevitable. Evidence of Iron toxicity was also observed on the fields with leave chlorosis, small panicles and low yield on rice farms. These observations were reported as evidence of iron toxicity by Mathias and Folkard (2005). The valley bottoms area were highly acidic. This could be attributed to high organic waste with slow rate of decomposition due to hydromorphic condition prevailing in such locations. This has been reported to cause immobilization of available nutrients and less availability of plant nutrients specially those nutrients which are soluble under high soil pH such as P and K (Mathias and Folkard (2005).

Management methods

Management practices such as burning of farm bye products and planting of arable crops with tree crops without adequate spacing are also contributing factors to soil fertility decline. In order to improve the soil quality, organic fertilizer or organic fertilizer fortified with inorganic fertilizers, suitable cropping system/combinations are recommended. Controlled fallow (Ande and Onajobi, 2009) which involve use of crops that produce high bio-mass rapidly should be included in the fallow such as Mucuna, Mexican sunflower and *Chromolaena odorata*. Audebert and Saahrawat (1996) observed that inclusion of legumes at the upland position above valley bottoms was capable

Table 2. Mean values of surface physical and chemical properties of soil from major cropping system

Land use	Sand (%)	Silt (%)	Clay (%)	Texture	BD gcm^{-3}	pH	Ca	Mg	K	Na	CEC	ESP%	%C	%0M	%N	P (mg/kg)	BS
							(Cmol/kg Soil)										
Rice	48	18.6	33.4	Clay loam	1.56	4.3	0.42	0.2074	0.15	0.48	1.255	38.25	0.1	0.17	0.01	4.38	58.78
Maize, Yam Cassava	19.6	24.4	56.00	Clay	1.60	5.7	1.23	0.366	0.17	0.41	2.177	15.12	0. 3	0.52	0.03	3.36	94.57
Agro – forestry	40	26.8	33.2	Clay loam	1.10	4.7	0.88	0.173	0.16	0.35	1.564	22.38	2.8	4.81	0.20	5.1	69.39
Maize, Yam Cassava	39	31.8	29.2	Clay loam	1.22	5.3	1.40	0.3675	0.145	0.145	2.057	7.05	2.01	3.46	0.20	5.44	93.42
Fallow	29	358	36.00	Clay loam	1.35	5.2	0.15	0.2805	0.19	0.35	1.835	19.07	1.27	2.18	0.12	4.67	87.59
Rice	31	34.2	34.8	Clay loam	1.50	4.6	1.44	0.517	0.18	0.18	2.317	7.77	1.26	2.17	0.13	5.02	46.61
Rice/sole Yam	23	41.2	35.8	Clay loam	1.31	4.8	0.68	0.46	0.77	0.77	2.68	28.73	2.66	4.58	0.27	3.63	80.64
Yam, maize Cassava/Okra	31	32.6	37.00	Clay loam	1.21	4.8	0.34	0.38	0.23	0.23	1.46	15.75	2.50	4.30	0.25	4.18	69.41
Fallow	35	26.4	38.60	Clay loam	1.40	5.1	0.48	0.38	0.25	0.25	1.36	18.38	2.21	3.80	0.22	3.38	89.76
Cassava, Maize, Potato	32	27.8	40.20	Clay	1.50	4.8	1.37	0.48	0.33	0.33	2.51	13.14	2.56	4.40	0.26	4.3	78.59

BD = Bulk density, CEC = Cation exchange capacity, BS = Base saturation, 0M = Organic matter.

Table 3. sub-surface physical and chemical properties of the soil from major cropping system in Ebonyin state.

Location	Landscape position	Cropping system	Sand (%)	Silt (%)	Clay (%)	Texture	pH	Ca	Mg	K	Na	CEC	ESP (%)	OC (%)	OM (%)	N (%)	P mg/kg	BS (%)
								(Cmol/kg Soil)										
1	Upland	Mixed cropping	46.4	30	23.6	Clay loam	4.9	1.84	0.38	0.4	0.15	2.76	5.43	0.05	0.09	0.01	3.36	82.21
2	Upland	Fallow	34	20.8	45.2	Clay	4.1	0.68	0.27	0.68	0.24	1.85	12.96	2.14	3.68	0.21	2.76	65.68
3	Upland	Agro-forestry	30	38.8	31.2	Clay loam	5.4	2.36	0.45	0.28	0.44	3.53	12.48	1.79	3.08	0.18	5.02	92.88
4	Midslope	Mixed cropping	30	38.4	31.6	Clay loam	5.2	1.32	0.32	0.21	0.32	2.17	14.75	0.92	1.52	0.92	4.105	93.53
5	Lower/valley	Mono cropping	34	9.2	56.8	Clay	4.5	1.41	0.062	0.15	0.15	1.77	8.48	1.9	3.27	1.9	5.1	69.32

Location: 1. Okorie (arable); 2. Oyinkwa (agro-forest); 3. Oyinkwa(arable); 4. Oyinkwa (fallow); 5. Azokpuru (rice). ESP = Exchangeable sodium percentage, CEC = Cation exchange capacity, BS = Base saturation, 0M = Organic matter.

of improving soil fertility and crop productivity.

Adequate use of fertilizers, appropriate tillage system (for example, plough across slopes) and minimum tillage on compacted soils should be encouraged to minimized erosion.

Conclusion

The results showed that cropping systems was the major contributing factor to soil degradation. Major land degradation indicators that were identified include improper crop combination and overcrowding of crops on heaps resulting in high competition for scarce soil resources and nutrient mining. Improper tillage system on the upland has resulted into soil loss. Poor yield of rice is attri-

Table 4. Indicators and criteria for land degradation for selected soil parameters

Location	Nitrogen	Phosphorus (P)	Potassium (K)	BS	ESP	Organic matter (OM)	BD gcm^{-3}
1	4	4	2	3	3	4	2
2	4	4	1	2	2	4	2
3	1	4	2	2	2	1	1
4	4	4	1	2	1	1	1
5	2	4	1	2	2	2	1
6	2	4	1	3	1	2	2
7	4	4	1	3	3	1	1
8	4	4	3	3	2	1	1
9	4	3	4	2	2	1	1
10	3	4	4	3	2	1	2

1 = None to slightly degraded, 2 = Moderately degraded, 3 = Highly degraded soil, 4 = Very highly degraded spoil. FAO (1979), Snakin et al. (1996).). ESP = ESP=Exchangeable sodium percentage, BS = Base saturation, BD = Bulk density.

Table 5. Scores for chemical and biological degradation of soils (Top soil).

Parameter	Degradation Level			
	1	2	3	4
Content of nitrogen %	>0.13	0.10 - 0.13	0.08 - 0.1	<0.8
Content of P (mg/kg)	>8	7 - 8	6 - 7	<6
Content of ESP (Increase by % of CEC)	< 10	10 - 25	25 - 50	> 50
BS (Decrease of saturation) 17 > 50%	>2.5	2.5 - 5	5 - 10	>.10
Organic Matter %	>2.5	2 - 2.25	1.0 - 2.0	<1.0
Soil bulk density (g/cm^3)	< 1.5	1.5 - 2.5	2.5 - 5	> 5

Locations 1. Okorie; valley bottom 2. Okorie; upland3. Oyinkwa; upland 4. Oyinkwa; upland upland 5. Oyinkwa; upland 6. Azokpuru; lowland /valley bottom 7. Abaomege; lowland /valley bottom 8. Abaomege; upland 9. Abaomege; fallow 10. Onu Ebonyin low land. ESP = Exchangeable sodium percentage, BS = Base saturation), CEC = Cation exchange capacity

buted to continuous cultivation of rice at the valley bottom with hydromorphic condition resulting to iron toxicity.

The government may play a crucial role by subsiding farm inputs and training of farmers through extension agents on good cropping and tillage systems. The major degradation issue in the area was low fertility while factors of land degradation include improper crop combination and impropertillage systems.

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Community Structure of Montane forest along the Altitudinal Gradient in Garhwal Himalaya, India

Minaxi Khali* and V P Bhatt

Department of Botany, Government PG College, Gopeshwar, Chamoli, Uttarakhand, India.

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The present study was done in sub-tropical and temperate Himalayan Forest of Saikot Reserve Forest, Kedarnath forest division in Chamoli district of Uttarakhand to understand the community structure and effect of altitudinal variation on structure and composition of the vegetation and to record the floristic diversity of the plants in the study area. The study area was categorized into four forest types on the basis of vegetation analysis, plant association or plant composition surveys, *viz* (1) Chir-Pine forest, (2) pine-oak forest (3) oak-pine-forest (4) oak-mixed forest. In the floristic study, a total of 58 species were recorded. Of the 58 plant species, 21 were tree, 11 shrubs and 26 herbs. The *Quercus leucotrichophora* forest was experiencing serious threat owing to human pressure and severe invasion of *Pinus roxburghii* which leads to loss of oak forest and development of pine forest.

Key words: Community structure, Saikot Reserve Forest, Garhwal Himalaya, altitudinal gradient, Montane Forest, aspects.

INTRODUCTION

Vegetation is a key factor in determining the structure of any ecosystem (Gaur, 1999; Bhatt and Purohit, 2009). Within a plant community, it determines microclimate, energy budget, photosynthesis, water regimes, surface runoff and soil temperature (Yadav and Gupta, 2006). The plant community of a region is a function of time and altitude. Slope, latitude, aspect, rainfall and humidity also play an important role in the formation of plant communities and their composition (Kharkwal et al., 2005).

The Himalayan vegetation ranges from sub-montane dry-deciduous forest in the foothills to alpine pasture above the timberline (Gaur, 1999; Bhatt and Purohit, 2009). The vegetation of Himalaya was intensively studied by Becking (1954). The literature on quantitative phytosociological work has been published from various parts of world (Cheema and Qadir, 1973; Beg and Khan, 1984; Ahmed et al., 2006; Ahmed et al., 2010; Khan et al., 2010a). Phytosociological characters differ among aspects and position, even in the same vegetation type. However, the different types of forest studies (structural and functional) in Garhwal Himalaya were shown by several workers such as Osmoston (1922), Puri (1960) and Champion and Seth (1968). Oak and other forest of Garhwal Himalaya has been studied for structure and

*Corresponding author. E-mail: bhattvp3@yahoo.com or khali15minaxi@gmail.com.

succession, and for impact of biotic stress by several workers like Tiwari (1979), Tiwari and Gupta (1982), Agrawal (1985), Rawat and Tiwari (1990), Joshi and Tiwari (1990), Mehta and Tiwari (1992), Bisht and Kusumlata (1993), Gaur and Bartwal (1993), Bhandari et al. (1995), Agarwal (1996), Mehta et al. (1997), Nautiyal et al. (1997), Bhandari et al. (1997), Bhandari and Tiwari (1997), Bhandari et al. (2000) and Bhandari et al. (2003). In Western Himalaya, the formation is represented by *Pinus roxburghii* (Mehta and Bhandari, 1997). But possibly, this is not the potential natural climax forest and its extensive occurrence today would not have been possible, had there not been continuing disturbances such as landslides, burning, deforestations, etc (Champion and Seth, 1968a, b). However, these forests are now stabilized over a large area and are regarded as a permanent feature of the mountains of western Himalaya.

The pine species is the principal tree of the lower and middle region of Garhwal region of western Himalaya (750-2100m asl) and constitutes the main source for fuel and shows a specific impact on the economy and the ecology of this region. Therefore, in the present study, an attempt has been made to quantify the species composition and structure in Saikot forest as very little information exists on this aspect in north western Himalaya.

MATERIALS AND METHODS

Study area

Geographically, the study area (Saikot Reserve Forest) is between 30° 21' 23.2'' to 30° 22' 47.6'' north latitude and 79° 17' 45.0'' to 79° 17' 44.2'' east longitude while attitudinally, it range from 800 to 2500 m asl and is the part of Gopeshwar range of Kedarnath Wild-life Forest Division of the district Chamoli.

Due to mountainous structure of the study area, it has varying altitude and relief which contribute greatly to variations in climatic condition. Owing to steepness of slopes, there are marked local effects due to differences in isolation. Temperature of the area fluctuates from time to time which makes specific micro-climate of the region. The average annual temperature of the region is 20°C. The maximum temperature is recorded in April to June (35°C) and minimum temperature in the valley recorded in the months of January-December (8-15°C).

The average annual rainfall of the area is 130 mm (based on the data of Gopeshwar Range). The humidity is inversely proportional to temperature. Where the temperature increases, these humidity will decrease. Relative humidity reaches almost the absolute humidity during July and August (80-92%). The minimum relative humidity reduces during the month of January-June and December (25-38%). The average annual relative humidity of study area is 55% which decreases with increase in temperature and altitude.

Soil

The soil in a region varies according to altitude, climate and vegetation in its texture and structure, therefore may be categorized into four types *viz.,* hard loamy soil, black loam soil, sandy loamy soil and sandy grey soil. Soil on the slope above 30°, due to erosion and mass wasting processes, are generally shallow and usually have very thin surface horizons. Such soils have medium to coarse texture.

Methodology

The vegetation was analyzed by laying the quadrate at different elevation (800 - 2500 m asl) and localities. The woody vegetation analysis was examined by using 10 × 10 m randomly placed quadrates on each sites on each sampling date. In each quadrate, all trees (>31.5cm cbh) and saplings or shrubs (10.50-31.40 cm cbh) were individually measured for circumference at breast height (cbh, that is, 1.37 m from the ground). For recording, the shrubs 5 × 5 m size quadrat were laid while for herbaceous vegetation, 1 × 1 m size quadrates were laid down randomly at each site separately. The vegetation data were analyzed quantitatively for abundance, density and frequency following the methods discussed by Curtis and Cottom (1956); species diversity ($\bar{H}$) was computed by Shannon-Wienner information function (Shannon-Wienner, 1963). Concentration of dominance (Cd) was computed by Simpson's index, (Simpson, 1949). β-Diversity was calculated by following the method of Whittaker (1975), while equitability (Ec) or species per log cycle index was determined following Whittaker (1972) and the community coefficient was calculated following Kulczynski (1927).

RESULTS AND DISCUSSION

The present study is based on extensive and intensive field study made during the months of September 2009 to September 2011 at various localities of Saikot Reserve Forest. The vegetation data were quantitatively analyzed for abundance, density and frequency according to the standard formulae.

A total of 58 plant species were recorded from the entire study area (Saikot Reserve Forest area Kedarnath forest division). Of 58 plant species, 21 were trees, 11 were shrubs and 26 were herbs. On the basis of plant association or plant composition surveys, the study area, can be categorized into four forest types *viz,* (1) Chir-Pine forest, (2) pine-oak forest (3) oak-mixed forest. The area can also be classified under four altitudinal gradients and aspects that is 800 - 1200 m asl (Site 1) at South-West aspect; 1200 - 1600 m asl (Site 2) at North-West aspect; 1600 - 2000 m asl (Site 3) at South-East aspect and 2000-2500 m asl (Site 4) at North-East aspect. The general features of the area are described in Table 1.

Site-wise (a total of 4 sites were studied in the area) of species distribution is presented in Table 2. Site 4 is relatively species poor than Sites 1 and 3. *Pinus roxburghii* is dominant species at Sites 1 and 2 and *Quercus leucotrichphora* dominates Sites 3 and 4. Although dominance was shared by number of species, no single species was found to compete with *Quercus leucotrichphora* in Sites 3 and 4; a climax species. On the basis of density, basal cover and importance value index (IVI), *P. roxburghii* was found to be most important and dominant species in Sites 1and 2; whereas *Q. leucotrichphora* dominated Sites 3 and 4 (Table 3). However, the presence of *P. roxburghii* in Site 3 is an indication of possible threat to the coexistence of climax and

Table 1. Site characteristics in the Saikot reserve forest area of Kedarnath forest division.

Site	Altitude (masl)	Slope (°)	Aspect	Forest types
Saikot (Site 1)	800-1200	20	SW	Chir-Pine (*Pinus roxburghii*)
Tangsa (Site 2)	1200-1600	25	NW	Pine-Oak (*Pinius roxburghii, Quercus leucotrichophora, Rhododendron arboreum*)
Devkhal (Site 3)	1600-2000	35	SE	Oak-Pine (*Pinius roxburghii, Quercus leucotrichophora, Rhododendron arboreum*)
Bamyala (Site 4)	2000-2500	50	NE	Oak-mixed (*Quercus leucotrichophora,Q. floribunda, Rhododendron arboreum, Myrica esculenta*)

SW,South-East; NW, North-East; SE, South-East; NE, North East.

Table 2. Distribution of plant species in different sites of the study area.

Site	Tree	Shrub	Herb	Total
1	7	6	11	24
2	7	4	7	18
3	9	3	8	20
4	6	4	6	16
Total	**21**	**11**	**26**	**58**

and associated species (Table 3). The late successinal and climax species *Q. leucotrichophora* in the region when disturbed severely by human pressure as grazing, lopping, felling and burning, changes the microclimatic conditions and invites the invading species like *P. roxburghii* in the area (Semwal and Mehta, 1996). The total basal area of the trees ranged from 1346.59 to 19427.64 m^2 h^{-1} and total density varied between 710 and 1140 h^{-1} across the Saikot Reserve Forest (Table 3). Total basal area and density of tree layer was reported in the range of 27-191.5 m^2 h^{-1} and 350 to 1787 plants h^{-1}, respectively, for various broad leaved, traditional conserved (sacred groove) and protected (Nanda Devi Biosphere Reserve) forests of Kumaun and Garhwal Himalaya (Saxena and Singh, 1982; Singh and Singh, 1987; Bhandari and Tiwari, 1997; Sinha and Maikhuri, 1998; Maikhuri et al., 2000). Higher values of basal cover density and lower values of density suggest that all sites are mature and climax in nature. Low tree density and less number of species in Site 4 reflect the forest is under high biotic pressure coupled with other abiotic factors which are not necessarily conducive for tree growth.

The number (density) of seedlings of any species can be considered as the regeneration potential of that species. From the density values (Table 3), it is concluded that the regeneration of oak (*Q. leucotrichophora*) in Site 4 is low, as compared to Site 3 and regeneration of *P. roxburghii* in Site 2 is low, as compared to Site 1, but not in that level of harsh conditions as has been pointed out by other worker in other studied area of Central Himalaya (Saxena et al., 1978; Ralhan et al., 1982; Tiwari and Singh, 1982; Saxena and Singh, 1984; Bankoti et al., 1986). The co-dominance of *P. roxburghii* with *Q. leucotrichophora* particularly in Site 3 (Table 3) is an indication that due to various anthropogenic (human) pressures oak regenerates in comparison with pine. Degradation of the oak forest through high anthropogenic pressure will provide appropriate conditions for the pine (an early successional, low nutrient demander and shade intolerant species) to invade, thereby posing a serious threat to the ecological balance of this region (Singh et al., 1984).

In Saikot Reserve Forest, species richness is very high in herb layers (present study) as compared to other broad leaved forests of Garhwal Himalaya (Bhandari and Tiwari, 1997; Bhandari et al., 1998). High species richness in herb layers may be due to relatively less developed canopy in these mature forests which permit sufficient sunlight to reach the ground resulting in the luxuriant growth of herb species (Table 3).

A/F ratio was used to assess the distribution pattern of the species. Vegetation was found in haphazard distribution by most of the species followed by regular distribution. Contagious distribution as observed in the present case has not been reported in tree layers from this part of the Himalaya (Table 4). It is interesting that the distribution pattern of shrub and herbs did not correspond with the distribution pattern of trees. Other workers (Saxena and Singh, 1982; Bhandari and Tiwari, 1997) findings for Central Himalaya are different. Greig-Smith (1957), Kershaw (1973) and Singh and Yadava (1974) have reported contagious distribution in natural vegetation. Preponderance of random distribution in tree and shrubs layers as compared to herbs layer reflects the dimension of biotic interferences in these strata.

In the present study, similarity values have been shown in Table 6. It was interesting to note that Site 1 was completely dissimilar to Site 3 and Site 4. This indicates that there is great influence of site characteristics (microclimatic variations, different aspect, soil composition and

Table 3. Phytosociological analysis of tree vegetation.

Site	Botanical name	F	A	D	TBC	IVI	A/F
	Tree						
	Pinus roxburghii	100	4.20	4.20	10594.97	171.40	0.0420
	Bauhinia semla	20	1.00	0.20	312.10	11.81	0.0500
	Terminalia alata	30	1.33	0.40	642.17	20.42	0.0444
	Toona ciliata	20	1.00	0.20	460.19	12.91	0.0500
	Bauhinia vareigata	50	1.00	0.50	382.32	26.56	0.0200
	Emblica officinalis	20	1.00	0.20	49.94	9.86	0.0500
	Engelharditia spicata	60	2.33	1.40	984.90	47.05	0.0389
		300	11.87	7.10	13426.59	300	
	Sapling						
	Pinus roxburghii	50	3	1.5	100.44	222.67	0.06
	Engelharditia spicata	30	1.333	0.4	23.22	77.329	0.0444
		80		1.90	123.65	300	
	Seedling						
	Pinus roxburghii	50	3.2	1.6	1.15	209.27	0.064
	Engelharditia spicata	30	1	0.3	0.14	54.255	0.0333
	Bauhinia semla	20	1	0.2	0.10	36.736	0.05
		100		2.1	1.38	300	
Site 1: Saikot	**Shrub**						
(800-1200)	*Woodfordia fruticosa*	60	2	1.2	38.22	74.149	0.0333
	Barberis asiatica	80	2.125	1.7	34.65	85.914	0.0266
	Rubus ellipticus	50	1.8	0.9	10.32	40.548	0.036
	Colebrookia oppositifolia	50	1.6	0.8	6.37	35.194	0.032
	Rubus niveus	40	1	0.4	3.85	22.747	0.025
	Solanum erianthum	50	1.2	0.6	17.25	41.451	0.024
		330		5.6	110.65	300	
	Herb						
	Cylindrica imperata	60	3	1.8	1.29	41.927	0.05
	Cymbopogon nardus	70	5.143	3.6	1.39	57.157	0.0735
	Themeda triandra	40	2.25	0.9	0.64	23.004	0.0563
	Artemisia nilagirica	50	3.8	1.9	0.61	31.073	0.076
	Heteropogon contortus	30	6.667	2	0.64	28.09	0.2222
	Dicliptera bupleuroides	40	2.25	0.9	0.64	23.004	0.0563
	Dioscorea bulbifera	40	2	0.8	0.25	16.95	0.05
	Thysanolacna maxima	40	2.5	1	0.32	19.147	0.0625
	Coleus froskei	30	0.667	0.2	0.06	8.3192	0.0222
	Poa annua	60	3	1.8	1.29	41.927	0.05
	Rumex hastatus	30	1	0.3	0.10	9.4176	0.0333
		490		15.20	7.23	300	

F, Frequency; A, Abundance; D, Density; TBC, Total Basal Cover; IVI, Importance Value Index; A/F, Abundance/Frequency ratio.

variation in altitudinal gradient) on the composition of vegetation. However, Mehta et al. (1997) reported similarity of 9.6 (between burnt grazed and unburnt grazed sites) to 74.9% (between unburnt protected and unburnt grazed sites) for the various forest sites under different management regimes in Garhwal Himalaya. Wikum and Wali (1974) and Saxena and Singh (1982) have pointed out the significant role of the site characteristics in plant distribution and similarity.

The values of diversity are presented in Table 5.

Table 3. Contd.

Site	Botanical name	F	A	D	TBC	IVI	A/F
	Tree						
	Pinus roxburghii	80	3.625	2.9	7817.07	88.668	0.0453
	Quercus leucotrichophora	100	4.4	4.4	8525.35	112.02	0.044
	Alnus nepalensis	40	1.25	0.5	308.28	16.489	0.0313
	Pyrus pashia	50	1.8	0.9	1569.55	29.403	0.036
	Lyonia ovalifolia	50	1.2	0.6	832.36	22.667	0.024
	Salix babylonica	20	1	0.2	146.75	7.7162	0.05
	Rhododendron arboretum	60	1.167	0.7	228.28	23.038	0.0194
		400		10.20	19427.64	300	
	Sapling						
	Pinus roxburghii	50	1.8	0.9	64.49	141.85	0.036
	Quercus leucotrichophora	50	1.2	0.6	32.29	98.09	0.024
	Alnus nepalensis	30	1.333	0.4	18.34	60.063	0.0444
		130		1.90	115.13	300	
	Seedling						
	Pinus roxburghii	50	1.6	0.8	0.57	145.17	0.032
Site 2: Tangsa	*Quercus leucotrichophora*	50	2	1	0.56	155.03	0.04
(1200-1600)		100		1.80	1.13	300	
	Shrub						
	Barberis asiatica	70	2.143	1.5	30.57	107.05	0.0306
	Rubus ellipticus	40	1.75	0.7	8.03	43.281	0.0438
	Colebrookia oppositifolia	60	1.167	0.7	5.57	48.102	0.0194
	Rubus niveus	60	4	2.4	19.11	101.57	0.0667
		230		5.30	63.28	300	
	Herb						
	Agrostis nervosa	40	3.5	1.4	0.70	33.075	0.0875
	Heteropogon contortus	70	5	3.5	1.11	63.851	0.0714
	Arundinela nepalensis	60	3.333	2	1.08	49.403	0.0556
	Bidens pilosa	60	2.667	1.6	0.86	42.856	0.0444
	Cynodon dactylon	50	4.2	2.1	1.04	46.834	0.084
	Ajuga bracteosa	40	5	2	0.64	36.486	0.125
	Taraxacum officinale	40	2.5	1	0.54	27.479	0.0625
		360		13.60	5.97	299.98	

F, Frequency; A, Abundance; D, Density; TBC, Total Basal Cover; IVI, Importance Value Index; A/F, Abundance/Frequency ratio.

Diversity is a combination of two factors, the number of species present, referred to as species richness and the distribution of individuals among the species, referred to as evenness or equitability. Single species populations are defined as having a diversity of zero, regardless of the index used. Species diversity therefore, refers to the variations that exist among the different forms. In the present study, Shannon-Wiener index of diversity was used. The value of diversity ranged from 0.8098 to 1.3266, 0.66737 to 1.2759 and 0.4377 to 0.5767, respectively, for trees, shrubs and herbs in Saikot Reserve Forest. The range of diversity in the present community forest stands is certainly lower than any other montane forests of Central Himalaya (Ralhan et al., 1982). Moderate amount of anthropogenic pressure on Saikot Reserve Forest is helpful in maintaining the higher species diversity. Such view was also expressed by Thadani and Ashton (1995) and Singh et al. (1997).

The value of beta-diversity ranged from 1.5789 to 2.3333, 1.5384 to 1.8181 and 1.0714 to 2.2916 for trees, shrub and herbs layers, respectively in Saikot Reserve Forest. These values are much lower than those

Table 3. Contd.

Site	Botanical name	F	A	D	TBC	IVI	A/F
	Tree						
	Quercus leucotrichophora	100	4.6	4.6	8912.87	111.07	0.046
	Lyonia ovalifolia	30	1	0.3	731.49	13.389	0.0333
	Pyrus pashia	100	2.7	2.7	3802.57	66.887	0.027
	Myrica esculenta	30	1	0.3	349.71	11.333	0.0333
	Rhododendron arboretum	40	2.5	1	1494.35	25.909	0.0625
	Phoebe lanceolata	50	2.2	1.1	1667.87	29.994	0.044
	Quercus floribunda	40	2	0.8	917.20	21.047	0.05
	Persea duthiei	30	1.333	0.4	571.85	13.406	0.0444
	Pinus roxburghii	20	1	0.2	123.31	6.9638	0.05
		440		11.40	18571.20	300	
	Sapling						
	Quercus leucotrichophora	70	1.571	1.1	78.82	151.54	0.0224
	Lyonia ovalifolia	60	1.167	0.7	37.68	96.939	0.0194
	Persea duthiei	30	1.333	0.4	19.90	51.525	0.0444
		160		2.20	136.40	300	
	Seedling						
Site 3: Devkhal (1600-2000)	*Quercus leucotrichophora*	100	2.6	2.6	1.86	181.41	0.026
	Lyonia ovalifolia	60	1.667	1	0.62	77.171	0.0278
	Pinus roxburghii	40	1.25	0.5	0.25	41.276	0.0313
		200		4.10	2.74	299.86	
	Shrub						
	Daphne papyracea	70	2.143	1.5	30.57	137.23	0.0306
	Pyracantha crenulata	40	1.75	0.7	22.29	79.99	0.0438
	Prinsepia utilis	60	1.167	0.7	16.11	82.785	0.0194
		170		2.90	68.97	300	
	Herb						
	Agrostis nervosa	100	5.7	5.7	1.82	58.985	0.057
	Arundinela nepalensis	90	7.333	6.6	2.10	63.693	0.0815
	Chrysopogon aciciculatus	60	3.333	2	0.64	25.145	0.0556
	Chrysopogon fulvus	50	4.6	2.3	1.65	35.081	0.092
	Hedychium spicatum	70	3.571	2.5	0.80	30.538	0.051
	Polygonum chinensis	70	4.857	3.4	1.08	37.032	0.0694
	Polygonum lanigerum	60	3.333	2	0.64	25.145	0.0556
	Achyranthis bidentata	60	2	1.2	0.86	24.359	0.0333
		560		25.70	9.58	299.98	

F, Frequency; A, Abundance; D, Density; TBC, Total Basal Cover; IVI, Importance Value Index; A/F, Abundance/Frequency ratio.

reported for oak and chir pine forests of Kumaun (Tewari and Singh, 1985) and Garhwal (Bhandari et al., 1997) Himalaya, respectively. Little difference in the beta-diversity indicates that the growth forms among different stands respond in similar fashion (Adhikari et al., 1991). These values of beta diversity show that the species composition varies across the slopes and aspects.

Values on concentration dominance (Cd) are similar to that reported by Whittaker (1965) and Risser and Rice (1971) ranging from 0.19 to 0.99 for certain temperate vegetation. The values of concentration of dominance (Cd) of the present study ranged from 0.2438 to 0.3993, 0.2008 to 0.4789 and 0.1318 to 0.1736, respectively for trees, shrubs and herbs layer for montane forests of

Table 3. Contd.

Site	Botanical name	F	A	D	TBC	IVI	A/F
	Tree						
	Quercus floribunda	40	3.25	1.3	2956.15	40.97	0.0813
	Q. leucotrichophora	80	3.25	2.6	5704.27	80.77	0.0406
	Neolitsea pallens	100	3.4	3.4	5770.25	94.915	0.034
	Acer ceasium	50	0.8	0.4	624.20	20.921	0.016
	Aesculus indica	60	1.833	1.1	1390.41	35.306	0.0306
	Symplocos ramosissima	50	1.2	0.6	1348.28	27.118	0.024
		380		9.40	17793.57	300	
	Sapling						
	Quercus floribunda	60	2	1.2	46.24	79.083	0.0333
	Acer ceasium	90	1.778	1.6	99.87	127.38	0.0198
	Aesculus indica	70	1.571	1.1	73.65	93.538	0.0224
		220		3.90	219.77	300	
	Seedling						
	Quercus floribunda	80	3.25	2.6	1.19	128.15	0.0406
Site 4: Bamyala (2000-2500)	*Q. leucotrichophora*	90	2.111	1.9	1.36	125.49	0.0235
	Acer ceasium	30	2.667	0.8	0.50	46.467	0.0889
		200		5.30	3.05	300	
	Shrub						
	Daphne papyracea	100	3.9	3.9	124.20	171.17	0.039
	Pyracantha crenulata	40	1.75	0.7	37.68	47.455	0.0438
	Rosa brunonii	80	1.125	0.9	18.34	55.861	0.0141
	Rosa macrophylla	40	1	0.4	6.24	25.512	0.025
		260		5.90	186.46	300	
	Herb						
	Arundo donax	90	4	3.6	1.79	43.621	0.0444
	Agrostis pilsula	90	4.111	3.7	1.95	45.16	0.0457
	Chrysopogon aciciculatus	100	4.7	4.7	3.37	61.443	0.047
	Eriophorum comosum	100	3	3	2.15	45.678	0.03
	Roscoea alpina	90	6.222	5.6	2.79	58.926	0.0691
	Polygonum chinensis	90	5.111	4.6	1.46	45.172	0.0568
		560		25.2	13.51	300	

F, Frequency; A, Abundance; D, Density; TBC, Total Basal Cover; IVI, Importance Value Index; A/F, Abundance/Frequency ratio.

Saikot Reserve Forest. Mean (Cd) values of 0.31 to 0.42 (Mishra et al., 2000) and 0.07 to 0.25 (Shivnath et al., 1993) were reported earlier from other parts of Indian Himalaya. The higher value of Cd in the forest growing on upper altitude was due to lower species richness (Bhatt and Purohit, 2009). According to Baduni and Sharma (1997), the Cd or Simpson's index was strongly affected by the IVI of the first three relatively important species in a community. Species diversity and dominance (Simpson index) are inversely related to each other (Zobel et al., 1976).

Conclusion

Generally, *P. roxburghii* forms the climax vegetation at lower altitude and *Q. leucotrichophora* forms at middle altitudes (1500-2000). However, due to the anthropogenic pressure, water table seems to reduce which is providing the suitable condition for *P. roxburghii* invasion at higher elevation and north aspect and this invasion leads to mixed community of oak-pine patches at north aspect and in higher elevation, indicating developmental stage of secondary succession. As a result, it may be

Table 4. Distribution pattern of different forest sites of Saikot reserve forest.

Site	Stratum	Distribution pattern (%)		
		Regular	Random	Contagious
Site 1 (Saikot)	Tree	14.29	85.71	
	Shrubs	33.33	66.67	
	Herbs	45.46	9.09	45.45
Site 2 (Tangsa)	Tree	42.86	57.14	
	Shrubs	25.00	50.00	25.00
	Herbs	14.29	14.29	71.43
Site 3(Devkhal)	Tree	22.22	66.67	11.11
	Shrubs	33.33	66.67	
	Herbs		12.5	87.5
Site 4 (Bamyala)	Tree	33.33	50.00	16.67
	Shrubs	50.00	50.00	
	Herbs	16.67	50.00	33.33

Table 5. Species diversity (H¯), concentration of dominance (CD) and equitability of different forest sites of Saikot Reserve Forest.

Site	(H¯)	CD	Equitability	β diversity
Saikot (I) Tree	1.3266	0.3993	5.294129	2.33333
Sapling	2.2177	0.6676	3.484131	2.5
Seedling	2.0263	0.6099	3.321928	3
Shrubs	0.66737	0.20089	9.548227	1.81818
Herbs	0.4377	0.1318	8.763037	2.29167
Tangsa (II) Tree	0.9490	0.2857	5.214453	1.75
Sapling	1.2239	0.3684	8.51831	2.30769
Seedling	1.6815	0.5062	20.6377	2
Shrubs	1.0632	0.3200	7.475054	1.73913
Herbs	0.5421	0.1632	12.86604	1.94444
Devkhal (III) Tree	0.8098	0.2438	6.60925	2
Sapling	1.2766	0.3843	6.828538	1.875
Seedling	1.5829	0.4765	4.189925	1.5
Shrubs	1.2759	0.3841	9.06363	1.76471
Herbs	0.5462	0.1644	10.80551	1.42857
Bamyala (IV) Tree	0.8173	0.2460	6.455646	1.57895
Sapling	1.3021	0.3920	18.43575	1.36364
Seedling	1.1379	0.3425	5.86071	1.5
Shrubs	1.5909	0.4789	4.044471	1.53846
Herbs	0.57679	0.17363	22.13477	1.07143

concluded that due to anthropogenic disturbances these forests are in unstable and in degrading stage therefore, immediate steps should be taken to save these ecologically and economically important forests and species. As per the Champion and Seth (1968) classification, the Saikot Forest broadly falls under the Himalayan moist

Table 6. Community coefficient of different forest sites of Saikot Reserve Forest.

Site 1	Site 1	Site 2	Site 3	Site 4
Tree	100	33.52	Dissimilar	Dissimilar
Sapling	100	20.52	Dissimilar	Dissimilar
Seedling	100	41.02	Dissimilar	Dissimilar
Shrub	100	60.55	Dissimilar	Dissimilar
Herb	100	13.88	Dissimilar	Dissimilar
Site 2				
Tree		100	25	26.53
Sapling		100	29.26	Dissimilar
Seedling		100	33.89	28.16
Shrub		100	Dissimilar	Dissimilar
Herb		100	17.30	Dissimilar
Site 3				
Tree			100	10.57
Sapling			100	Dissimilar
Seedling			100	51.06
Shrub			100	50
Herb			100	21.21
Site 4				
Tree				100
Sapling				100
Seedling				100
Shrub				100
Herb				100

temperate forests category.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Hybridization between non-indigenous *Oreochromis niloticus* and native *Oreochromis* species in the lower Kafue River and its potential impacts on fishery

Ian Bbole[1]*, Cyprian Katongo[2], Andrew M. Deines[3], Overson Shumba[4] and David M. Lodge[4,5]

[1]National Aquaculture Research and Development Centre, Mwekera, Kitwe, Zambia.
[2]Department of Biological Sciences, University of Zambia, Lusaka, Zambia.
[3]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556. 574-631-4159, United States of America.
[4]School of Mathematics and Natural Sciences, Copperbelt University, Kitwe, Zambia.
[5]Environmental Change Initiative, University of Notre Dame, United States of America.

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In the Kafue floodplains, Zambia, a study was done to determine whether meristic and/or other morphological metrics could distinguish putative hybrids (based on color patterns) between non-indigenous *Oreochromis niloticus* (Nile tilapia) and two native species of *Oreochromis andersonii* (three spot tilapia) and *Oreochromis macrochir* (green head tilapia) from the pure parental strains. We also surveyed local fishermen to document their knowledge and beliefs on the spread of *O. niloticus* in the last decade, the occurrence of hybridization, and any changes in catch per unit effort of these and other species. A sample of fifty fish specimens were collected for morphometric and meristic data using gill nets and seine nets, and augments by specimens purchased from the catches of local fishermen. A full standard multi-filament net with mesh sizes ranging from 25 to 150 mm in increments of 12.5 mm mounted in a fleet was used. The seine net used was of 25 mm mesh size, approximately 100 m long and 2 m high at the pocket. We analyzed the morphological and meristic data using the program STATISTICA. Our results confirm that: in the last decade, *O. niloticus* has spread throughout the Kafue floodplains from its initial site of introduction near the eastern end of the Kafue floodplain; putative hybrids between *O. niloticus* and *O. andersonii* cannot be distinguished from parental species based on morphometric or meristic traits; and survey results of local fishermen indicate that they have experienced increases in catch per-unit- effort of *O. niloticus* while simultaneously experiencing decreases in catch per-unit-effort of native tilapia.

Key words: Indigenous knowledge (IK), hybridization, native, invasive species, morphometrics, meristics, Kafue River.

INTRODUCTION

In Zambia, subsistence and commercial fish catches are important for social and economic development of communities and the nation. The fisheries sector contributed 1.24% to the Gross Domestic Product (GDP) between 2002 and 2007 (Musumali et al., 2009) equivalent to US$51- 135 million per annum. The fishery sector plays a pivotal role in the economy through employment especially to the rural poor and provides an affordable animal protein source for many households in Zambia. Over 300,000 people in Zambia earn their income directly as fishers and fish farmers or indirectly as traders, processors and other service providers (Musumali et al., 2009).

The production of fish from aquaculture has been on a steady increase globally. Aquaculture has been noted to be one of the fastest growing food-producing systems in the world (Ahmed and Lorica, 2002). Tilapia culture has seen a rapid global expansion especially in developing countries (El- Sayed, 2006) and it is practiced in over 100 countries globally (FAO, 2004). Due to the environmental challenges associated with terrestrial agriculture, tilapia culture has been promoted as an important source of animal protein that could provide food security especially for developing countries (Canonico et al., 2005). In 2010, approximately 32 million tonnes of farmed tilapia was produced worldwide (FAO, 2012). *Oreochromis niloticus* (Nile tilapia) and its hybrids comprise approximately 80% of the total tilapia aquaculture production in the world (FAO, 2006). While global tilapia production contributes substantially to economic development, the escape or release of tilapia into the waters outside their native range is a concern for a number of reasons (Canonico et al., 2005).

Introduced tilapia species, typically of the genus *Oreochromis,* which establish feral populations can cause adverse ecological impact such as the competition with the native species for food, breeding sites and habitats (Canonico et al., 2005). They may also cause eutrophication especially in intensive tilapia culture due to release of excreta into the water and spread pathogens and parasites to native fish species (Starling et al., 2002; Dabbadie and Lazard, 2010). These factors may cause reduction in biodiversity of the receiving ecosystem and population of native fish species. When formerly isolated tilapia species are brought into contact through introduction mediated by aquaculture, hybridization with closely related native species may occur and have negative genetic impacts by the production of hybrids in the population (D'Amato et al., 2007). This may threaten or even eliminate pure native wild populations that are important genetic resources for future aquaculture breeding programs (Lind et al., 2012). Furthermore, reduced fecundity has been reported in some hybrids, which may eventually lead to lower fish yields (Amarasinghe and De Silva, 2010).

In addition to aquaculture, tilapias have been introduced for biological control of aquatic weeds and insects, as bait for capture fisheries and for re-stocking of capture fisheries (Canonico et al., 2005). Among the tilapias introduced in many parts of Africa, *O. niloticus* (Nile tilapia) and its hybrids account for over 90% of the production (Senanan and Bart, 2010). Since its introduction in Lake Victoria in the 1950s, it has spread to Eastern and Southern Africa as far as the Limpopo River in South Africa due to intentional introduction especially for aqua-culture purposes (Wise et al., 2007). Several reports indicate that in many freshwater systems in Africa where Nile tilapia has been introduced and has established itself and is productive, it has completely eliminated the native *Oreochromis* species (Shipton et al., 2008). *Oreochromis niloticus* has become invasive in many water and wetland systems as a result of intentional introductions or escapes from aquaculture facilities (Gupta et al., 2004). In Lake Victoria, for example, the introduction of Nile tilapia completely eliminated *Oreochromis esculentus* within a period of 30 years (Goudswaard et al., 2002; Wise et al., 2007). The establishment of feral populations of Nile tilapia in an ecosystem is almost impossible to control and the only way to reduce the impact of this species is to prevent its entry into new freshwater habitats (Wise et al., 2007).

According to Schwanck (1995), *O. niloticus* ((locally known as 'Namadama' (fish from the dams) or 'Wamunyima' (name of the manager at Zambia Sugar Estate who kept the fish) was imported together with *Oreochromis aureus* from the University of Stirling in 1982 and in 1988 and introduced into the Kafue catchment area by the Zambia Sugar Estate. However, by 1990 *O. aureus* had disappeared because it did not breed well. At the same time, Kafue Fisheries Ltd cultured the *O. niloticus* further down the Kafue River. Utsugi and Mazingaliwa (2002) reported that during the flood of 1989, fish escaped into the Kafue River. The first catches of Nile tilapia were reported in 1992 in the Kafue River (Shipton et al., 2008), and were spreading in 1994 (Schwanck, 1995).

The presence of introduced *O. niloticus* in the Kafue River, and its hybridization with at least two native species (*Oreochromis andersonii* and *Oreochromis macrochir*), has been documented with genetic analysis (Deines et al., 2014). In particular, individuals with both the vertically barred tail of *O. niloticus* and the lateral black spots of *O. andersonii* were confirmed to be hybrids between the two species (Figure 1D). In this paper, we extended these results by: surveying local fishermen to qualify their assessment of the occurrence of *O. niloticus* and hybrids in the Kafue floodplain, and their assessment of any changes in catch-per-unit-effort of *O. niloticus* and native species; and test whether putative hybrids (with both the barred tails and lateral spots) can also be distinguished

*Corresponding author. E-mail: ibbole@gmail.com.

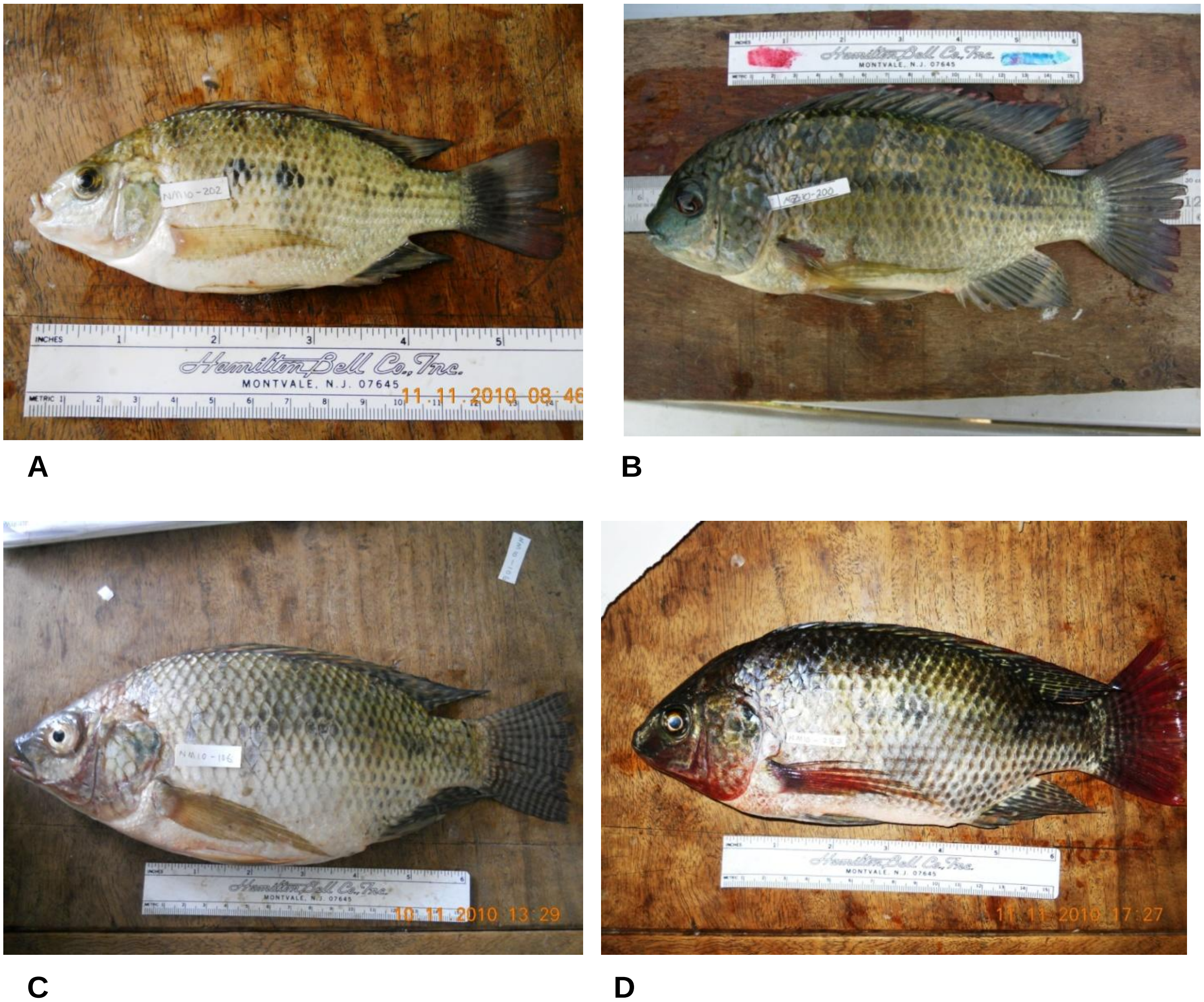

Figure 1. Fish species: (A) *Oreochromis andersonii*, (B) *Oreochromis macrochir*, (C) *Oreochromis niloticus*, (D) Putative hybrid (*O. andersonii* x *O. niloticus*).

from parental species on the basis of morphometric or meristic traits.

METHODS

Study area

The Kafue River system supports an extensive fishery in Zambia. It covers a stretch of about 1,576 km and an area of 152,000 km^2. The Kafue flood plains themselves are 250 km long and 60 km wide covering an area of approximately 6,500 km^2 (Figure 2) (Chabwela and Mumba, 1988). The Kafue fishery is mainly exploited by artisanal fisherfolks using fibre glass and dug-out canoes. They use a variety of fishing nets such as gill nets and seine nets as well as hooks to catch fish. Their nets range in size from 1" – 7" (2.54 cm – 17.78 cm) mesh. Illegal fishing methods are very common such as the use of explosives and 'Kutumpula' bashing water to drive fish towards the nets.

The fish species composition of ten most important fish species in the Kafue fishery for the period 1985 to 2005 included: *Clarius gariepinus, Schilbe intermedius, Marcusenius macrolepidotus, Serranochromis angusticeps, Serranochromis macrocephalus, Oreochromis andersonii, Clarius ngamensis, Synodontis kafuensis, Hepsetus odoe* and *Sargochromis condringtoni* (Nyimbili, 2006). Over the years, there has been a decrease in catch per unit effort of these species.

Interviews of fishermen

With the approval of the Zambian Department of Fisheries, and following the approach of Schwanck (1995), we conducted interviews on 14 fishermen in 7 of the sampling sites (Choongo, Musa Gate, Chitobolo, Namwala, Nyimba, Kapongo and Cheba) (Figure 2) to qualify their assessment of how the occurrence of *O. niloticus* changed over time, and any changes in catch-per-effort of *O. niloticus* and native species. Fishermen were selected with the aid of local authorities who identified them as experienced and

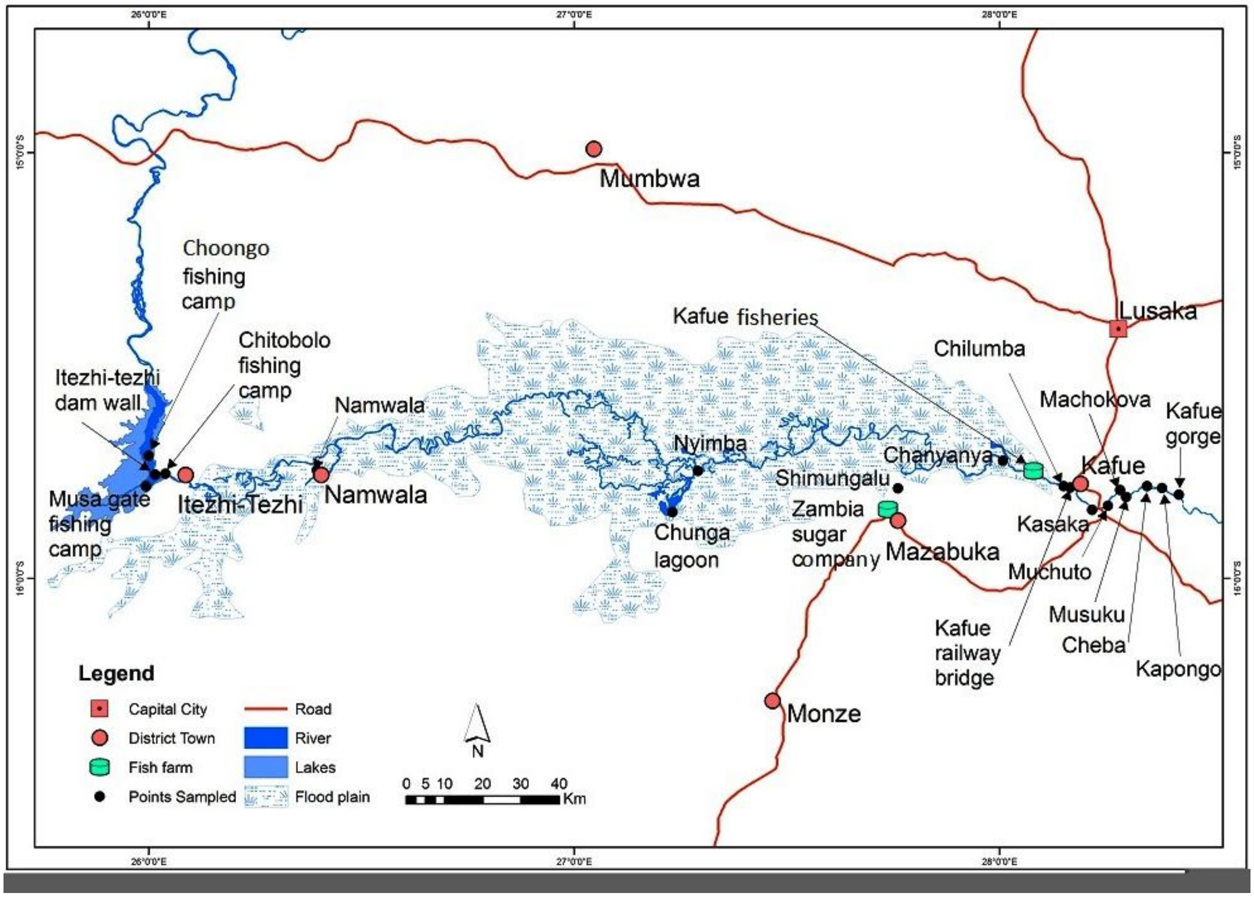

Figure 2. Fishing camps and actual sites sampled on the Kafue floodplains.

having been in the industry for more than 10 years. The questionnaire was administered in the local dialect and the responses were written by the questioners who included the author and the local fishery personnel. The responses used in this paper were: the person's fishing location, fishing method, type of species targeted, what species they have observed to be increasing or decreasing. The questionnaires were also designed to find out how much information the fishermen had on *O. niloticus*. The information on *O. niloticus* included; whether they have caught the species before, which year they made their first catch if they have caught it before, the catch per effort of the species and whether they have observed any changes in the catch per effort of native *Oreochromis* species since its introduction.

Fish sampling

Sampling was done in six sites and additional samples were collected from fishermen in four other different sites. *Oreochromis niloticus*, *O. macrochir* and *O. andersonii* were identified using Skelton (2001). The putative hybrids were identified as individuals with the distinct vertical stripes of *O. niloticus* on the caudal fin and either the three or four conspicuous black mid-lateral blotches of *O. andersonii* or the steep rounded head profile of *O. macrochir* (Trewavas, 1983) (Figure 1).

A total of 50 individuals were sampled for morphometric measurements and meristic counts from 10 sampling sites along the length of the Kafue floodplains (Figure 2 and Table 1). *Oreochromis niloticus* had previously been detected at some of the sampling sites (Schwanck, 1995). Sites at Choongo and Musa Gate are above the Itezhi-tezhi Dam where there is no indication that *O. niloticus* is present.

Samples of *Oreochromis* species were collected using a standard gillnet survey, seine nets and from fishermen during the 2010 low-water season (August-September). Gillnets set was carried out for three consecutive nights in six sampling sites. A full standard multi-filament net with mesh sizes of 25, 37.5, 50, 62.5, 75, 87.5, 100, 112.5, 125, 137.5 and 150 mm mounted in a fleet was used. Each net used measured 45 m and the fleet used was, therefore 495 m long. The setting of the nets was done at approximately 6 pm and removed the following day at 6 pm.

Fish samples were supplemented using beach seine nets at Choongo and Namwala sites. The seine net used was of 25 mm mesh size, approximately 100 m long and 2 m high at the pocket and was dragged by six men over a period of about 20 to 30 min. Two hauls were made consecutively. Other fish samples were collected from the local fishermen after carefully studying their catches. Most of their catches were mainly from seine nets. Collections supplemented from fishers took place at Chitobolo, Chilumba, Kasaka and Kapongo (Figure 2).

A colour photograph of each fish sampled was taken. Each fish was then individually placed in a polythene bag and preserved in 10% formalin in 20 L buckets according to species for further processing as described below.

Morphometric measurements and meristic counts

Morphometric measurements and meristic counts of each fish were obtained according to methods of Barel et al. (1977) and Snoeks

Table 1. Fish sampled for morphometric measurements and meristic counts

Specie	Sampling site	Total length range (mm)	Total fish sampled
Oreochromis andersonii	Choongo, Namwala, Chunga lagoon, Nyimba, Shimungalu, Chanyanya, Kasaka, Kapongo	141-274	18
Oreochromis macrochir	Namwala, Chunga lagoon, Nyimba, Shimungalu, Chilumba, Kasaka	69-380	14
Oreochromis niloticus	Chitobolo, Chunga lagoon, Nyimba, Shimungalu, Chilumba	122-256	12
Putative *O. andersonii* x *O. niloticus*	Namwala, Shimungalu, Kapongo	192-246	6

(1994). These measurements were taken using a vernier calliper to the nearest 0.01. The meristic counts were done under a dissecting microscope.

The morphological measurements taken were: Lachrymal depth (LacrD) defined as preorbital depth (POD), snout length (SnL), lower jaw length (LJL), premaxillary pedicel length (PPL), cheek depth (ChD), horizontal eye diameter/eye depth (EyD), vertical eye diameter/eye length (EyL), inter-orbital width (IOW), head width (HW), head length (HL), standard length (SL), body depth (BD), dorsal fin base length (DFB), anal fin base length (AFB), pectoral fin base length (PFL), predosal distance (PrD), prepelvic distance (PrP), prepectoral distance/preventral distance (PrV), preanal distance (PrA), caudal peduncle length (CPL), caudal peduncle depth (CPD)

The meristic counts taken included: the upper and lower jaw teeth numbers (UJT and LJT), inner tooth rows, dorsal fin spiny rays (DSPIN) and dorsal fin soft rays (DSOFT), anal fin spiny rays (ASPIN) and anal fin soft rays (ASOFT), upper and lower lateral line scales (ULL and LLL), lateral line to anal fin scales (LLA), pelvic/ventral fin scales (PV), number of scales between the dorsal fin and the upper lateral line (DLL), scales around the caudal peduncle (CP) and cheek scales (CK).

Data analysis

Interviews

The information provided by the fishermen on the questionnaires was analysed to reveal the general understanding the fishermen had on introduction of *O. niloticus*. We found this was possible because Nile tilapia were easily distinguished by fishermen from other tilapia by the presence of stripes on the caudal fin, a characteristic not possessed by other species of tilapia (Trewavas, 1983).

Morphometric and meristic data handling

The morphometric and meristic data were analyzed using STATISTICA Version 5 (StatSoft, Inc., 1997). The raw morpho-metric data were transformed into logarithms and subjected to PCA to extract principle components.

The data were then used to plot relationships among the fish species based on principal component (PC) 2 and PC 3. Principal component 1 was not used in morphometric analysis because in multivariate allometry PC 1 represents the size and shape as all the characters are correlated positively with this component (Lleonart et al., 2000). Meristic count data where analyzed based on PC 1 and 2. This is because meristic counts are not influenced by allometry on PC 1. The factor loading was used to identify which of the morphometric and meristic characters were causing most variations among the fish.

RESULTS

Distribution of *Oreochromis* species in the Lower Kafue River

The invasive species *O. niloticus* was found along the whole 250 km stretch of the Kafue floodplains. It was however, absent in the stretch of the Kafue River upstream of the Itezhi-tezhi Dam during this study. The local fishermen also confirmed that they had not caught *O. niloticus* in the Kafue River upstream the Itezhi-tezhi Dam wall. They also indicated that their first catch of *O. niloticus* on the Kafue River below the Itezhi-tezhi Dam wall was around the year 2002. The information from the local fishermen indicated that by 2004 *O. niloticus* was present in all areas in the Kafue floodplain.

During this study, *O. niloticus* catch was higher than both *O. andersonii* and *O. macrochir* catches for almost all the sampling done using both seine and gill nets. Our observations of a subset of catches by fishermen were consistent with the pattern reported by the fishermen: *O. andersonii* was the least frequent in most of the catches. In Shimungalu, Chunga lagoon and Nyimba, *O. andersonii* was very rare.

Survey results

Twelve of the fishermen interviewed were using dug-out canoes as fishing vessels while two were using small engine boats. They were using both seine nets and gill nets with mesh sizes ranging mainly between 1-3½ inches (25.4 to 88.9 mm).

All the fishermen interviewed were aware of the pre-sence of *O. niloticus.* By 2004, *O. niloticus* had spread upstream as far as the Itezhi-tezhi Dam and downstream to Kapongo (Kafue Gorge Dam) (Table 2). All except one of the fishermen interviewed reported observing high catch-per-effort of *O. niloticus* in their fishing areas, and the species is among the three most targeted fish species,

Table 2. Results from interviews of fishermen conducted during the study. Sites are ordered from west to east (see Figure 2)

Site	Number of fishermen interviewed	Type of fishing vessel	Type of nets used	Mesh size (mm)	Year started fishing	First catch of *O. niloticus*
Choongo	1	Dug-out canoes	-	-	-	No *O. niloticus*
Musa gate	1	Dug-out canoes	-	-	-	No *O. niloticus*
Chitobolo	1	Dug-out canoe			-	2002
Namwala	5	Dug-out canoes	Seine nets Gill nets Hooks	1¼" - 3½"	1979, 1994, 2004	1996, 2001, 2004
Nyimba	4	Small engine Fibre glass Dug-out canoes	Seine, Gill Rod and line	1.0" - 6.0"	1976, 1992, 1994	1994, 2000, 2001
Cheba	1	Dug-out canoes	Seine	1¼"	1978	1995
Kapongo	1	Dug-out canoes	Seine	1¼"	1982	2004

and among the three most commonly caught species together with *Tilapia rendalii* and *Barbus* species (Figure 3).

Oreochromis niloticus was one of the fish species that fishermen reported increasing in catch-per-effort together with Redbreast tilapia (*T. rendalii*) (Figure 4). Fishermen identified *O. andersonii* and *O. macrochir* as fish species for which the catch-per-effort had declined (Figure 4). Fishermen reported catching fish possessing features of both *O. niloticus* and *O. andersonii,* suggesting potential hybridization between these species.

Morphometric and meristic results

A total of 50 specimens sampled from the Kafue floodplain were assigned species identification according to morphology and colour patterns: *O. niloticus, O. andersonii, O. macrochir* and putative hybrids between *O. niloticus* and *O. andersonii*.

Overall, the PCA results provided clear discrimination among the three parental species, but the putative hybrids always clustered with *O. niloticus*. PCA results distinguished *O. andersonii* from *O. niloticus* on PC 2 although a few individuals from both species overlap. *Oreochromis andersonii* is similar to *O. macrochir*. On PC 3, *O. macrochir* is different from *O. andersonii* but similar to *O. niloticus* and the putative hybrids.

The three morphometric characters that showed most significant variations between putative species along PC 2 included: Premaxillary pedicel length (0.4007), lower jaw width (-0.3492), and caudal peduncle length (-0.2876). The variations between species along PC 3 were mainly due to: vertical eye diameter/eye lengthy (-0.2987), horizontal eye diameter/eye depth (-0.2762) and anal fin base length (0.2357) (Figure 5).

The meristic results indicate that the fish species could only be separated on PC 2 where *O. macrochir* is different from both *O. andersonii* and *O. niloticus* and the putative hybrids. Again, the putative hybrids clustered together with *O. niloticus*.

The three meristic characters that showed the most significant variations along the PC 1 include: Upper lateral line scales (0.8293), anal fin soft rays (0.7688) and lower lateral line scales (0.6555). On PC 2 they include: Number of scales between the anal fin and upper lateral line (-0.3971), caudal peduncle scales (-0.3249) and dorsal fin soft rays (0.3038) (Figure 6).

DISCUSSION

Indigenous knowledge of the distribution of *O. niloticus*

This study has revealed that *O. niloticus* is now distributed along the whole of the 250 km stretch of the Kafue floodplains. Schwanck (1995) survey found *O. niloticus* confined within a stretch of 75 km between Muchuto and Kachola in the eastern region of the floodplain. However, he rightly predicted that *O. niloticus* would expand its range upstream, being finally restricted by the Itezhi-tezhi Dam wall. The sampling done at Choongo upstream before the dam wall and the study of the fishermen catches at Musa Gate also before the dam wall revealed no presence of *O. niloticus*. This means that the dam wall has acted as a geographical barrier preventing further spread of the *O. niloticus* upstream.

The sampling conducted during this study at different sites and the study of tilapia catches from fishermen along the floodplain showed higher catches of *O. niloticus* followed by *O. macrochir* and *O. andersonii* which was least. A study by Chikopela et al. (2011) in three major habitats of the Kafue floodplains revealed that *O. niloticus* had the highest index of relative importance (IRI) and contributed most to the diversity and evenness of the floodplain as compared to *O. andersonii* and *O. macrochir*. *Oreochromis niloticus* had not only established its popu-

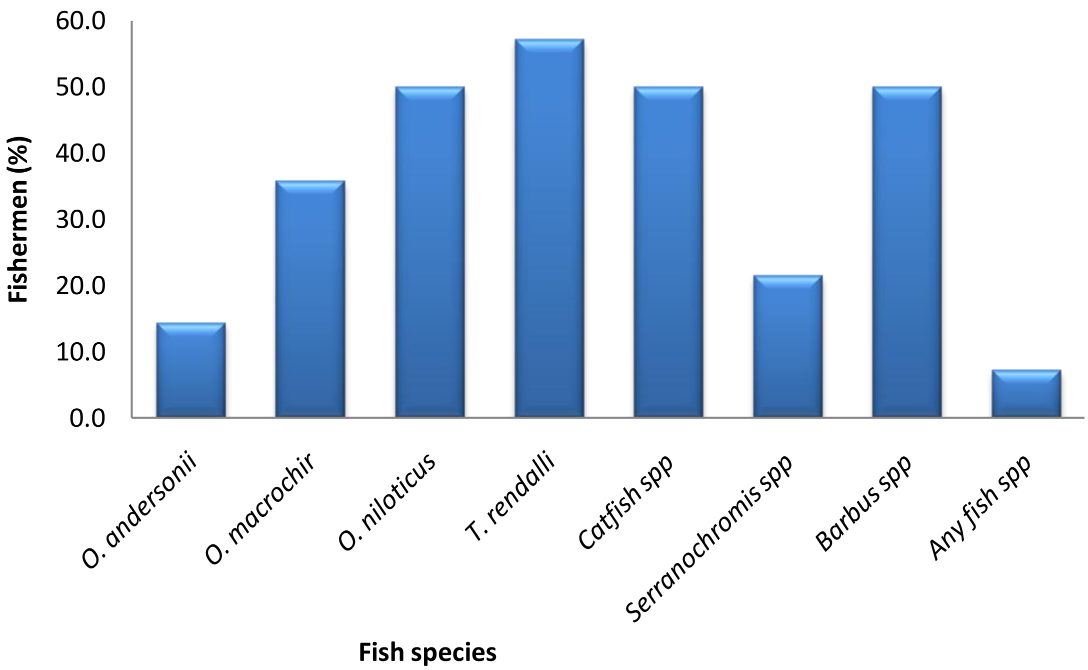

Figure 3. Fish species caught by local fishermen interviewed.

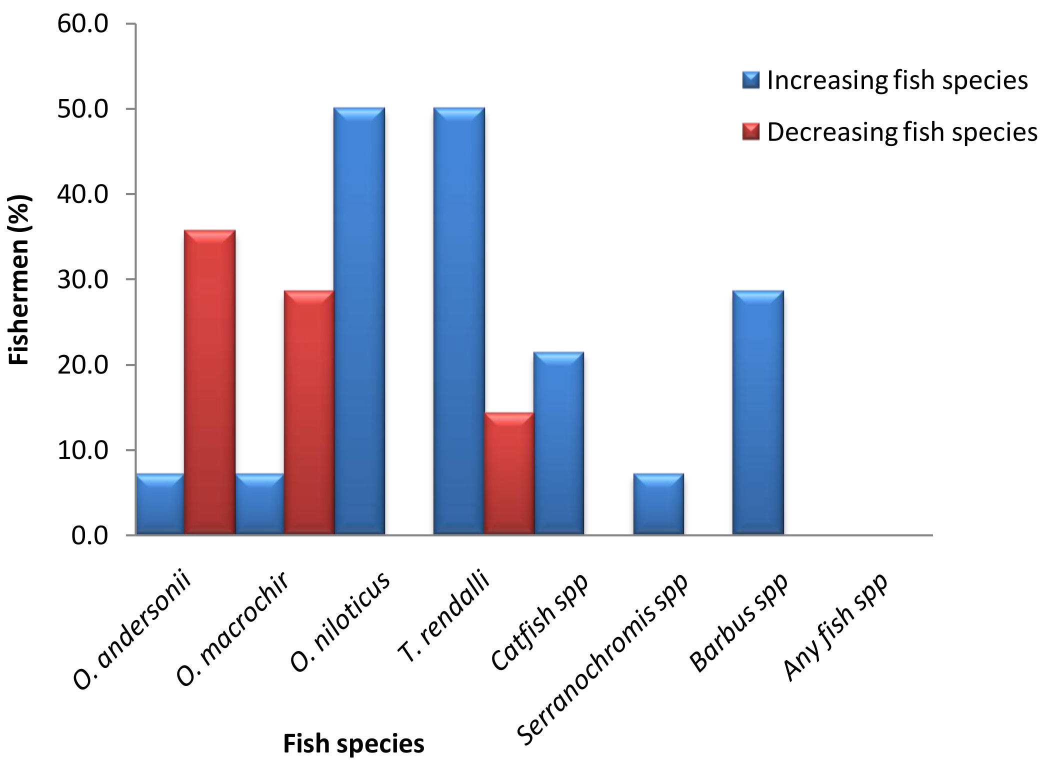

Figure 4. Increasing and decreasing fish species as reported by the local fishermen interviewed.

lation in the Kafue but has dominated the numbers among the mouth brooding tilapiines (Chikopela et al., 2011). The local fishermen interviewed have also reported a decrease in catch-per-effort of both *O. macrochir* and *O. andersonii* catches since the introduction of *O. niloticus*. This seems to indicate that the introduction of

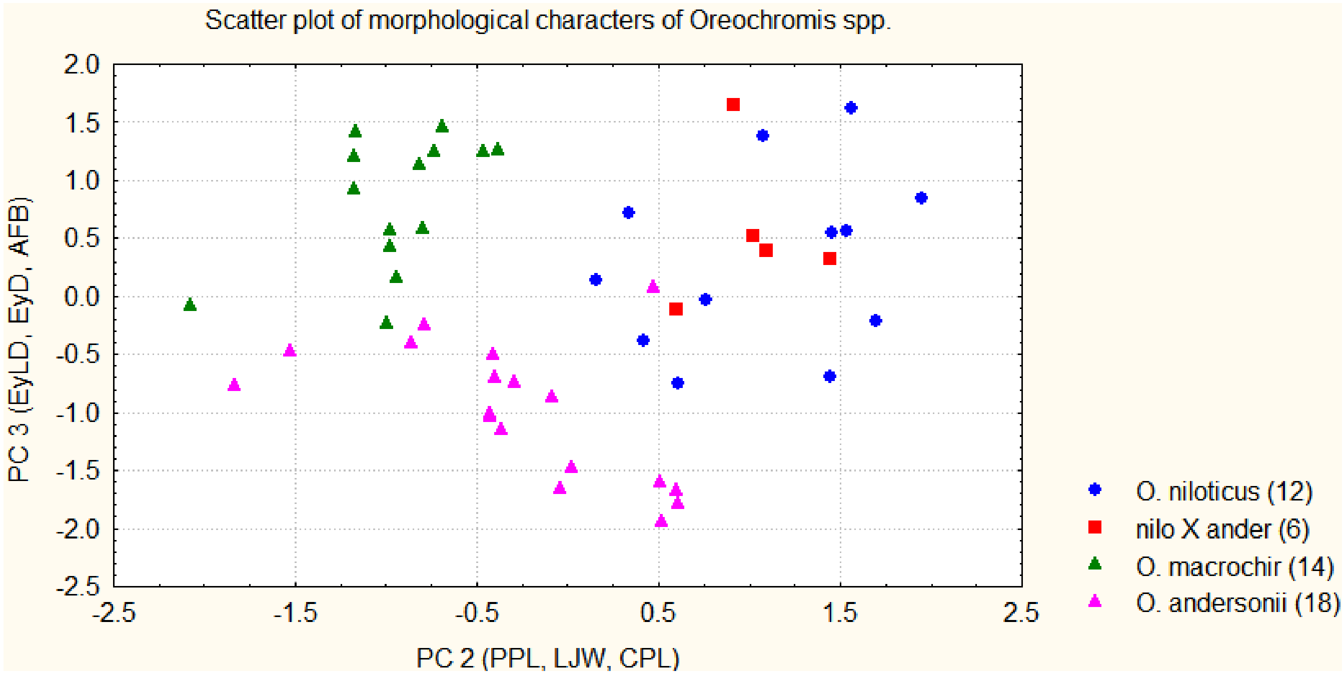

Figure 5. PCA plot of morphometric characters of *O. niloticus, O. andersonii* and *O. macrochir* including suspected hybrids. Each data point represents an individual fish. PPL = Premaxillary pedicel length, LJW = lower jaw width, CPL = caudal peduncle length, EyLD = vertical eye diameter/eye lengthy, EyD = horizontal eye diameter/eye depth, AFB = anal fin base length.

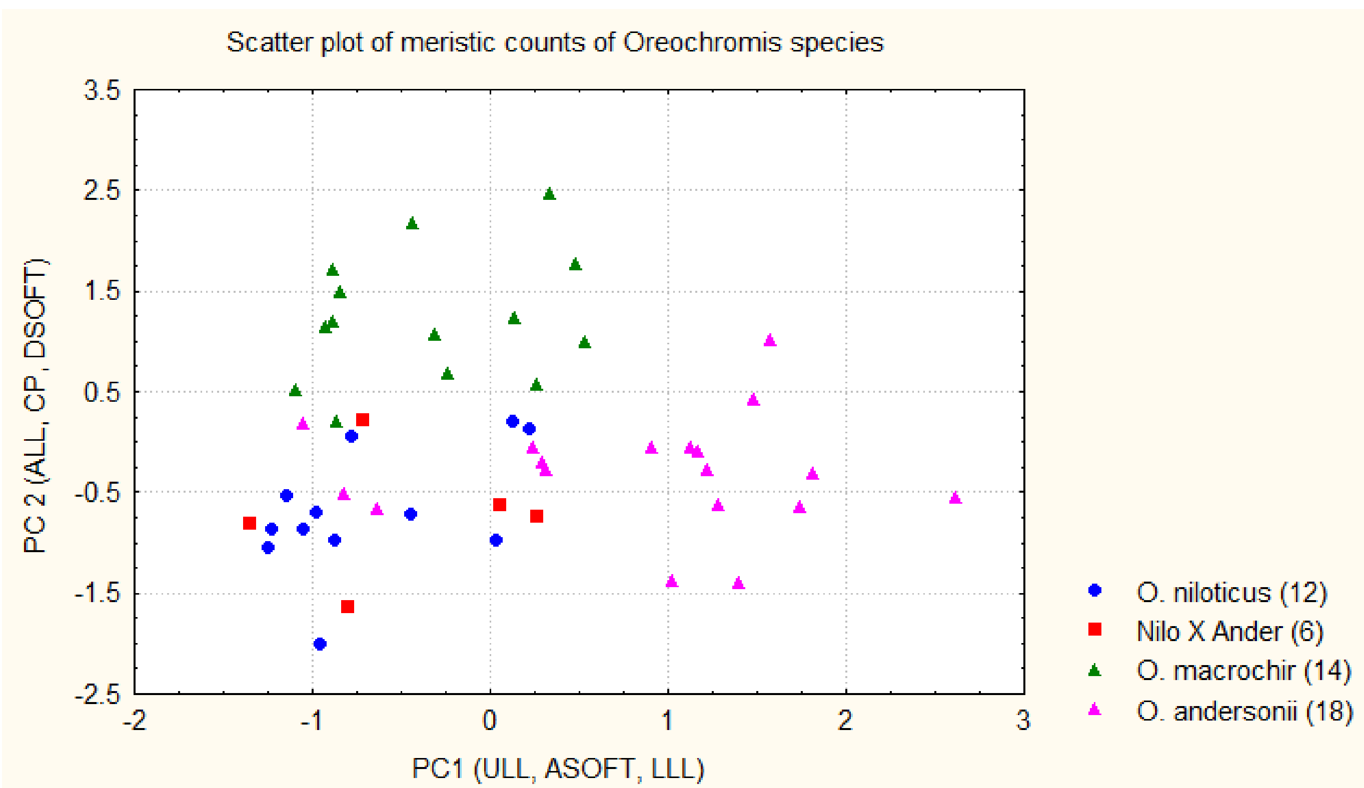

Figure 6. PCA plot for meristic counts of *O. niloticus, O. andersonii* and *O. macrochir* including suspected hybrids. Each data point represents an individual fish. ULL = Upper lateral line scales, ASOFT = anal fin soft rays, LLL = lower lateral line scales, ALL = number of scales between the anal fin and upper lateral line, CP = caudal peduncle scales, DSOFT = dorsal fin soft rays.

the non-indigenous *O. niloticus* may have an impact on the population of other related species like *O. macrochir* and *O. andersonii*. This assumption may not be conclusive since sampling was not done throughout the year. In addition, fishing pressure, introduction of non-indigenous crayfish (*Cherax quadricarinatus*) which three of the fishermen reported, or other unmeasured factors could contribute to the decline in catch-per-effort of native fishes during the spread of *O. niloticus*.

Taken at face value, however, the reduction in catches of *O. andersonii* reported by fishermen, and its apparent absence in areas closest to the point of release of *O. niloticus* like Shimungalu in Mazabuka indicate a strong negative interaction with O. *niloticus* leading to the establishment of the non-indigenous species at the expense of the indigenous species. The probable explanation of this is the potential of *O. niloticus* to hybridize with other *Oreochromis* species followed by the extinction of the hybrids leaving only pure *O. niloticus* strains (Shipton et al., 2008). The takeover is usually preceded by a period of introgressive hybridization (Schwanck, 1995).

Some fishermen reported that they had seen *O. niloticus* individuals possessing features of other native *Oreochromis* species, especially the three or four spots of *O. andersonii* consistent with the hybridization confirmed by Deines et al. (2014).

While the socio-economic impact arising from the introduction of *O. niloticus* in the Kafue floodplains was not investigated fully in this study, the fishermen interviewed had different views on the effect of *O. niloticus* on their catches. Among the fishermen interviewed, 41.7% of them felt that *O. niloticus* has affected their catches of native tilapia while 33.3% said it did not. Another 25% of fishermen interviewed did not know whether there was an effect or not. The fishermen claimed that the introduction of *O. niloticus* has reduced their sales of native tilapia which are one of the most preferred fish in Zambia which also fetch a higher cost. This is because they are not able to catch large quantities of native tilapia as they used to before *O. niloticus* was introduced. Some felt that their income has reduced because *O. niloticus* cannot be preserved for a longer time as compared to the native tilapia. Some fishermen interviewed confirmed that they have resorted to the use of bad fishing methods like 'bashing' the water to drive fish to the set nets in order to catch native tilapia.

These results suggest that establishment and spread of *O. niloticus* throughout the Kafue floodplains may pose a threat to the ecology and existence of local *Oreochromis* species. The invasion of *O. niloticus* may also pose a challenge to the sustainability of the fishery and eventually the livelihoods of people dependent on the fishery. This is because a fishery which usually exploits variety of species is more likely to be sustainable than one which depends on only a few species (Dulvy et al., 2000). As long as catch-per-effort of *O. niloticus* remains high, the fishery may be maintained, but perhaps be moving to an *Oreochromis* monoculture of *O. niloticus*.

Hybrids identification based on morphometrics and meristics

Our analysis demonstrated that morphometric and meristic traits are no use in distinguishing hybrids of *O. niloticus* x *O. andersonii* from parental individuals. Thus where genetic analysis is not possible future studies on hybridization will have to continue to rely on colour patterns to identify hybrids, while acknowledging that not all hybrids and backcrosses possess the mixed colour patterns (Deines et al., 2014). However, any estimate of the prevalence of hybridization will be poor without genetic analysis, especially because genetic analysis from specimens on the Kafue floodplain revealed hybrid mixture of all the three *Oreochromis* species, while colour patterns had suggested hybridization involving only *O. niloticus* and *O. andersonii* (Deines et al., 2014).

Implications

The issue of food security, dietary and nutritional needs of people in local communities illustrates a concern that goes beyond the potential ecological and genetic impacts on biodiversity of escaped tilapias from aquaculture facilities into receiving ecosystems such as dams, rivers, wetlands or lakes (Seaman and Bart, 2010). As noted by the WorldFish Centre (2009), fish is an important food for over 400 million Africans, contributing essential proteins, minerals and micronutrients to their diets. Zambia is no exception. Fish in Zambia is estimated to provide about 40% of the animal protein intake. It therefore, plays an important role in the food and nutrition of the Zambian people more especially the urban poor and people living with HIV and AIDS (Musumali et al., 2009).

Taking the case of the Kafue flood plains, fishing is a major mainstay industry for local communities. The observation in this study of the possibility of hybridisation and its potential threats to native fish species that provided food security, employment, economic security, and self-sustenance for this largely rural part of Zambia raises serious concerns. In this fishery, just as in other fishery areas, fishing provides income almost all year round to men, women and youths who cannot find other sources of income.

The results of this study suggest that the presence of *O. niloticus* in the Kafue floodplain has had an impact on the indigenous *Oreochromis* species. Fishermen reported a reduction in the catch-per-effort of the native *Oreochromis* species which are commercially important and which are widely favoured by most Zambians. The poverty situation is already apparent in the Kafue floodplains as some of the fishermen interviewed were requesting the government to send them relief food, especially during the fish ban period between February and March annually. This is because they cannot catch enough to sustain their families.

Recognising the risks inherent with any species introduction, and the particular risks quantified here on the Kafue floodplain (loss of genetic identity of native tilapia, reported loss of income by fishermen), FAO (1996) advocates that the Precautionary Principal should apply, a consideration relevant to the Zambian Department of Fisheries and similar agencies in other countries to consider with regard to potential future introductions of *O. niloticus*. FAO (2006) recommends that the governments reduce risks of adverse impacts of introduction on capture fisheries to establish corrective or mitigating procedures in advancement of actual adverse effects, and to minimize unintended introduction to wild ecosystems and associated capture fisheries. Loss of native species can lead to loss of genetic diversity necessary for future food security and poverty reduction (Lind et al., 2012). According to Musumali et al. (2009), about 68% of the Zambian population live below the poverty line. It is more rampant in the rural area were about 81% live in poverty.

The poverty and food insecurity in Zambia stems from the over reliance on rain-fed agriculture, and associated effects of frequent unfavourable climatic conditions, along with inadequate incomes, access to markets and transport facilities to enable the transfer or purchase of food. This is compounded by low economic diversification into sectors such as fisheries that could supplement crop production. Food security entails the access to enough amounts of safe, nutritious and quality food. For communities in this study, wild fishing is an important if not the most vital activity by which local families meet their food needs and achieve economic security. Anything that disturbs this vital source of nutrition and economic livelihood is likely to embolden poverty, imperil food security, and thwart self-sustenance aspects of their food security.

Conclusion

This study has revealed that the local fishermen throughout the Kafue floodplain are very aware of the introduction of *O. niloticus* in their fishery. They have reported an increase in catches of *O. niloticus* and associate this increase with the reduction in catches of native *O. andersonii* and *O. macrochir*. Moreover, they have observed apparently hybrid fish that combine characteristics of *O. niloticus* and native *Oreochromis* species.

There are differences in morphological characters of *O. niloticus*, *O. andersonii* and *O. macrochir* consistent with their classification as different species. However, the hybrids between *O. niloticus* and *O. andersonii* could not be distinguished from *O. niloticus* based on the quantifiable morphometric and meristic measurements.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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