ABUNDANCE, DISTRIBUTION AND GENETIC ASSOCIATION OF ANAMBRA WAXBILL (*Estrilda poliopaeria*, L) IN NIGERIA.

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ABSTRACT

Endemic species are in constant danger of extinction due to natural and anthropogenic causes. The Anambra Waxbill *Estrilda poliopareia* is an endemic bird species with a small population reported from different locations in Anambra, Bayelsa and Delta States of Nigeria. Faced with ecological and evolutionary pressures, there is an increasing need to evaluate the status of the refugia populations. This study aims to evaluate the abundance, distribution and genetic status of Anambra Waxbill in Nigeria. Surveys have so far been carried out at historical and reported sites of Anambra Waxbill from November 27, 2014 to October 10, 2015. Point transects and mist-netting were employed to census birds and vegetation characteristics measured afterwards. A total of 104 individuals were trapped to collect blood and feather samples for molecular analysis. During this survey, 467 individuals of Anambra Waxbill were recorded in the study sites. The abundance of Anambra Waxbill was not influenced by the vegetation parameters measured; however, the occurence of the species was significantly influenced by the grass height, percentage litter cover and number of stumps. The results suggest that forest clearings with increase in grass height and decreased litter cover may provide opportunities for Anambra Waxbill to colonize new habitats. The results also suggest a need to balance exploration and utilization of resources in the Niger Delta region, with sound understanding of ecological needs of other taxa on a regional scale.

AFRICAN BIRD CLUB

INTRODUCTION

The Anambra Waxbill *Estrilda poliopareia* (Reichenow, 1902; 1907; Chapin, 1950) is an endemic Afrotropical resident bird species (Elgood et al. 1994; Fry and Keith, 2004; Bannermann, 1949; Hall and Moreau, 1970; Clements, 2000; Sibley and Ahlquist, 1990; Serle, 1957). It is listed as Vulnerable in the International Union for Conservation of Nature's Red listing criteria for threatened species (IUCN, 2015). To date, very little is known of its current population status, breeding biology and habitat ranges (Collar and Andrew, 1988; BirdLife, 2016). The species has recently been reported in Benin Republic, in addition to the historic records from Nigeria (Ash, 1990; Collar and Stuart, 1985; Roux and Otobotekere, 2005; Butchart, 2007; Plomp *et al*., 2012). Currently, it is not known whether the population in Benin Republic is different from the one in Nigeria or whether the population in Nigeria expanded their range from south west Nigeria into Benin Republic. Previously, Anambra Waxbills were reported in small flocks of 20 individuals or more in the southern parts of Nigeria (Plomp *et al*., 2012). They are commonly found in areas with long grasses along rivers, lagoons, sandbanks, swamps, edges of open deciduous trees; yam and cassava farmlands (Roux and Otobotekere, 2005). As granivores, they feed on grass seeds especially *Kyllinga, Finbristylis*, *Cerastium, Digitaria* and *Panicum* spp., and seeds of other weeds associated with disturbances. Nests are built on stands of *Penisetum purpurem* during the breeding periods starting in April (Roux and Otobotekere, 2005). Although, it is likely that the species breeds year round, as nesting activities have been observed all year round (Coker *Pers Obs*.).

The taxonomic controversy surrounding the Anambra Waxbill and the Arabian Waxbill, *Estrilda rufibarba*, as well as Fawn-breasted Waxbill, *Estrilda paludicola* has persisted probably due to the similarity among these species (Clement et al. 1993; BirdLife, 2009). Thus, it remains an aspect of the species' biology requiring molecular resolution for the proper classification and genetic status . Only molecular techniques are adequate to resolve taxonomic issues where morphological information is considered insufficient (Dowsett and Dowsett-Lemaire, 1993). Where Anambra Waxbill exists within reportedly suitable habitats, the population is usually small, hence the possibility that there are particular limiting factors yet unknown (Birdlife, 2016). In general, there is very little knowledge about the persistence of the pocket populations within Nigeria. Thus, a series of field trips were conducted during this study between November 27th, 2014 and October 10th, 2015 to investigate the abundance, distribution as well as the degree of relatedness within and between populations of Anambra Waxbill; in order to improve our knowledge of the ecology and genetic status of the species.

MATERIALS AND METHODS

Study sites

Field work was carried out at Tombia (04.999°N, 06.261°E) and Agudama (05.011°N, 06.264°E). I also visited 2 reported historical sites (south-western Nigeria) and 7 other locations contiguous to Tombia and Agudama (in the Niger delta) between November 27th, 2014 and October 10th, 2015 (see Figure 1): Topo (06.397°N, 02.939°E) and Badagry (06.401°N, 02.878°E), Akaibiri (04.983°N, 06.274°E), Sabegreia (05.031°N, 06.247°E), Amassoma (04.969°N, 06.119°E), Polaku (05.036°N, 06.275°E), Oko – Amakom (06.134°N, 06.751°E), Ogulagha (05.383°N, 05.317°E), Okolabia (05.050°N, 06.243°E), and Kaima (05.116^{0}N, 06.302°E).

The mean annual rainfall of the study areas ranges from 2,000 – 2,500 mm near the coast, and 1,500 – 2,000 mm further north. The region is drained by River Niger, Cross River, River Imo, Ogun-Osun River systems and River Kwa-Ibo. The vegetation is defined by the climate of the area with mixture of rainforest, deciduous forest and parkland forest. Common floral species are *Cola* spp., *Mausoria altissima*, *Nesogordaonia papaverifera*, *Pterygota* spp., *Triplodriton sceroxylon*, *Sterculia* spp., *Antiaris africana*, *Ficus* spp., *Milicia excelsa*, *Celtis* spp., *Cylicodiseus gabunensis*, *Gossweilrodendron balsamiferum*, *Piptamiastrum africanum.* Saline tolerant grass species include *Paspalium vaginalum, Sporobotus robustus, Sporobotum virgincus, Eriochoa fatmensis, Eragrostis domingensis* and *Cyprus articulatus*; while species such as *Panicum repens, Imperata cylindrica* and *Pycretus polystachyos* are common in non-saline areas along the coastal line (Swaine et al., 1979). Menceae include *Entandrophragma* spp., *Guarea* spp., *Khaya ivorensis* and *Lovoa tricholioides*.

Large mammals such as the threatened Pygmy Hippopotamus, *Hexaprotodon liberiens*, are now greatly diminished in the region. Other rare fauna include Sclaters' Guenon, *Cercopithecus sclateri*, Crested Chameleon, *Chamaeleo cristatus*, African Manatee, *Trichechus senegalensis*, together with several eastern and western species of duikers and primates.

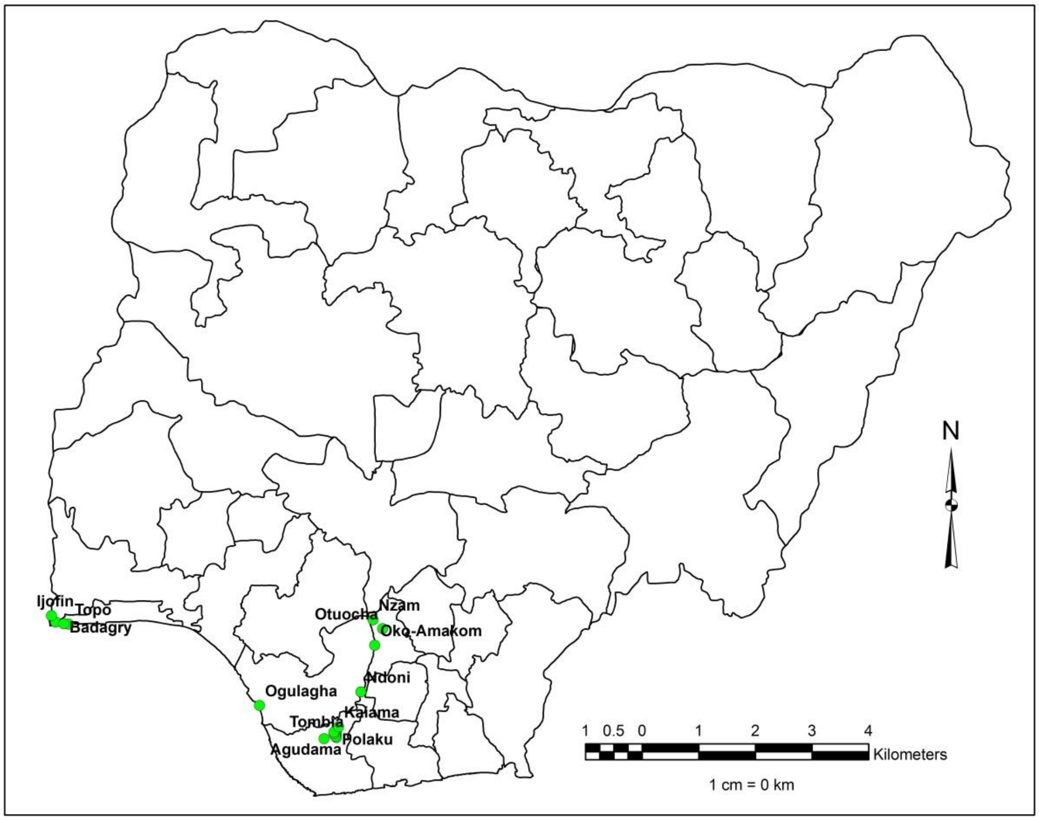


Figure 1: Map of study sites in southern Nigeria.

Conservation efforts in the region are quite minimal; however there are some reserves in the study area including: Anambra Forest Reserve (194 km^2), Mamu River Forest Reserve (70 km^2), Osomari Forest Reserve (115 km^2), Akpaka Forest Reserve (296 km^2) and Stubb Creek Forest Reserve (80 km^2).

Bird survey method

Point transect

Bird surveys were carried out using point transect method to determine the distribution and abundance of Anambra Waxbill. Point transect combines point count techniques and typically involves walking along a transect and stopping at regular intervals to record birds.

A total distance of 47,800 m and 239 points were surveyed across all the study sites. Surveys were carried out in the morning between 6.00 am and 10.00 am, and afternoon between 3.30 pm and 6.30 pm. Point count stations were located at least 200 m apart from each other along transects so as to reduce the risk of double counting and also provide a more representative data and coverage of the survey areas. At each point, three minutes was allowed for the birds to settle from observer disturbance. Bird data such as number of individuals seen perched, heard and flying were recorded in a field notebook. A total of 10 minutes was spent at each point (Bibby, 2000). Bird identification was aided by the use of a pair of binoculars (Opticron, magnification 8 x 40), and a Helm field guide (Borow and Demey, 2008).

Point transects were adopted because they are suitable for counting conspicuous birds, and can easily be adapted for a single species survey. It is also more efficient in terms of data collected per unit effort, and appropriate in areas where access is poor like in waterlogged and marshy environment or where the habitat is fine-grained. Also the method has special value in habitat studies, especially that it allows habitat characteristics to be measured at such points (Buckland *et al*, 1983; Bibby *et al*, 2000).

The points were systematically laid out, avoiding habitat features such as gullies, quick sandbars, mud flats, swamps and deep water bodies. The choice of this method was therefore predicated on increased bird observation, topography and vegetation (Wright, 2002).

Mistnetting

Anambra Waxbills were trapped with mist nets at four sites: Tombia (n=78), Oko-Amakom (n=13), Agudama (n=6) and Ogulagha (n=7). Mist-netting was done in the morning (6.00 – 10.00 am) and afternoon (3.00 – 6.30 pm). All trapped individuals were fitted with Safring aluminium rings and aged based on the plumage and bill colouration.

Coker,2015

Plate 1: Setting up of mistnet at study site by the Field Assistant

Coker,2015

Plate 2: Anambra Waxbill trapped in a mistnet

The ageing protocol in the APLORI ringing guide was adopted. The bird's age was scored as follows: 1, nestlings; 2, age unknown; 3, juveniles with gape and blackbill; 4, complete adults; 5, juveniles with transitional bill colour; and 6, adults older than 2 years old usually identified by old adult plummage moulting into adult (Cox, 2011; Borrow and Demey, 2001). Morphometric data, namely, body mass to the nearest 0.1 g, were taken using a spring weighing balance, fat scored as 0 representing no visible fat to a maximum of 9 representing full fat (Bairlein, 1995), wing length to the nearest 0.1 mm (Svensson, 1992), tarsus length, total head length (Green, 1980) were recorded. Moult score of flight feathers were recorded according to Ginn and Melville (1983); an old feather scored as 0, growing feathers on a scale from 1– 4 and a new full grown feather scored as 5.

Coker, 2015

Plate 3: Adult Anambra Waxbill

Collection of blood samples

One hundred and four individual (n=104) Anambra Waxbills were sampled. Upon capture, the brachial vein on the underside of the left wing was punctured with a hypodermic needle. 25 µl of blood was collected with a 0.25 mm capillary tube. The blood sample collected was added to 5ml of 95% ethanol in an eppendorf sample vial. The mixture was screw sealed and shaken thoroughly to prevent the blood from coagulating. All samples were properly labelled with the ring number of the bird from which the sample was collected, the date of collection and location. Samples were stored at room temperature during the field work, thereafter they were tranferred to the A.P Levetis Ornithological Research Institute, Jos, Nigeria (APLORI) where they were stored in a refrigerator at 0° C.

Ten (10) blood samples were sent to Prof Michael Sorenson at Boston, USA for molecular analysis to determine the phylogenetic relationship between Anambra Waxbill, Fawn-breasted Waxbill, Arabian Waxbill and other Waxbills (see Figure 3).

Twenty (20) blood samples were also sent to Dr Talatu Tende at the Molecular Ecology and Evolution Laboratory at Lund University in Sweden for molecular analysis to determine the degree of relatedness between and within populations of Anambra Waxbill. The remaining blood samples are still in store at APLORI.

Molecular analysis

DNA Extraction from the samples sent to Molecular Ecology and Evolution Laboratory

DNA was extracted from each of the twenty blood samples of the Anambra Waxbill. 5µL 10 mg/ml proteinase K, 250µl SET-buffer and 7µl 20% SDS were added to the blood samples in 2mL eppendorf tubes, then incubated at 55°C in a water bath overnight. Thereafter 250µl of 4M Ammonium acetate (NH_4Ac) solution was added to each of the samples. These samples were again properly mixed and allowed to rest on the bench for 60 minutes, while shaking the mixture at 15 minute intervals.

The samples were centrifuged at 13,000 rpm for 10 minutes to allow any pellet to precipitate. The supernatant containing DNA, soluble proteins and buffer solution was poured into new labelled 1.5 ml tubes, and two volumes of 100% ethanol were added to each sample in order to wash the DNA. The mixture was thoroughly mixed and centrifuged for 15 minutes at 13,000 rpm. The supernatant was carefully poured into a new labelled 1.5 ml tube avoiding the side where the DNA pellet was. 1 ml (1000 µl) of ice cold 95% Ethanol was added using a pipette and the mixture was thoroughly vortexed. The tubes were centrifuged at 13,000 rpm for 15 minutes; these were organized with the labelling facing out from the centre. The supernatant was poured off with care not to lose the DNA-pellet. 1 ml of 70% Ethanol was added to rinse the pellet, and this was poured out immediately.

The samples were air dried in a cupboard overnight. 100 µl ddH_2O was then added and vortexed. The samples were left to dissolve in the fridge overnight, thereafter the DNA concentration of each sample was determined with a nanodrop.

Polymerase Chain Reaction

All amplifications were done using 2X Qiagen multiplex PCR kit in 10 µl reaction volume containing 5 µl Qiagen multiplex PCR buffer mix; 0.2 µM forward primer (Applied Biosystems), 0.2 µM reverse primer, 2.6 µl water and 2 µl of DNA extract with a hot start at 95°C for 15 minutes. The thermal profile consisted of 35 cycles with a "touch-down" procedure where the annealing temperature was lowered 0.5^{o}C per cycle, starting from 55^{o}C, until a temperature of 46^{o}C was reached. Additional 25 cycles were ran at a constant annealing temperature of 46^{o}C. The denaturation step was at 95^{o}C for 30 seconds, annealing for 45 seconds and extension at 72^{o}C for 30 seconds. A final extension step of 10 minutes was added to the last cycle.

A blank control (reagents only) from DNA extraction process was included in all PCRs to monitor for contamination. The results of the PCR were evaluated by electrophoresis using 2% Agarose gels and GelRed™ (Biotium) staining. Positive samples were further sequenced using the forward primer (BigDye sequencing kit; Applied Biosystems, Foster City, CA, USA) in an ABI Prism® 3100 capillary sequencer (Applied Biosystems). The final processes of these analyses are still pending.

Collection of feathers

A second outer feather was carefully plucked off from the right wing (Karau, 2008). This was placed in separate envelopes, and labeled with location name, species name, ring number, date, and age. These feathers collected will be used to determine heavy metal loads as indices of environmental threats in the Niger Delta due to oil pollution . The use of feathers to determine heavy metal is ideal because elements are sequestered into growing feathers , thereby reflecting local contaminant level and can easily be detected (Burger,1993). The feather samples are kept in the refrigerator at APLORI for further analysis.

Vegetation measurement

At each point surveyed, a 10 m x 10 m quadrat was taken and the following parameters estimated: number of big trees (trees with circumference at breast height, (CBH) of more than 50 cm), number of small trees (trees with CBH of 10 - 50 cm), number of stumps, number of saplings, number of grass with seed, number of flowering plants, number of fruiting plants, height of grass, and perch height of Anambra Waxbill when sighted. Four 1 m x 1 m quadrats were taken randomly within the 10 m x 10 m quadrat and used for estimation of litter cover to the nearest 5% by eye (Manu, 2002). The patch size surveyed was estimated from the total length of point transect covered and the total width apart per study site.

Advocacy Visits and Public Awareness

Public awareness campaigns were conducted in Tombia, Agudama, Akaibri and Amassoma. Advocacy visits to Traditional Councils and Elders fora were embarked upon to inform the communities of the conservation importance of the species. Educational materials were produced and distributed to 18 primary schools and 12 secondary schools in the host communities.

Statistical Analysis

All data collected were compiled using Microsoft Excel 2007. Analyses were done in R version 3.1.3 (R Core Development Team 2015) and SPSS version 16.0.

Zero-altered Negative Binomial models which are able to take the zero-inflated data into account were used to investigate the relatonship between Anambra Waxbill abundance and the measured habitat variables in a two part model i.e. to model the count data (ZAP model) as well as the summarised binomial data (ZANB model) respectively (ZANB model AIC = 557.6, LRT: $\chi 2$ (1) = 387, $p < 0.001$). Density was estimated as the number of Anambra Waxbills sighted per proportion of patch surveyed.

Results

A total of 467 individuals were sighted and recorded during the survey and 104 individuals were mist-netted for blood and feather samples collection (see Table 1).

Table 1: Abundance and Density of Anambra Waxbill across study sites

Site	Latitude(°N)	Longitude(°E)	Patchsize (Km^2)	Number sighted	Estimate Density (/km^2)	Number trapped	Number retrapped
Akaibiri	4.983	6.274	0.53	22	41.5	0	0
Polaku	5.036	6.275	3.24	6	1.85	0	0
Agudama	5.011	6.264	1.19	64	53.78	6	0
Tombia	4.999	6.261	1.66	143	86.14	78	4
Amassoma	4.968	6.122	0.45	20	44.44	0	0
Sabegreia	5.031	6.247	0.77	8	10.39	0	0
Kaiama	5.116	6.302	3.84	6	1.56	0	0
Oko-Amakom	6.134	6.751	12.75	153	12.00	13	0
Ogulagha	5.383	5.317	1.61	50	29.43	7	0
Topo	6.397	2.939	20.3	0	0	0	0
Badagry	6.401	2.878	13.6	0	0	0	0

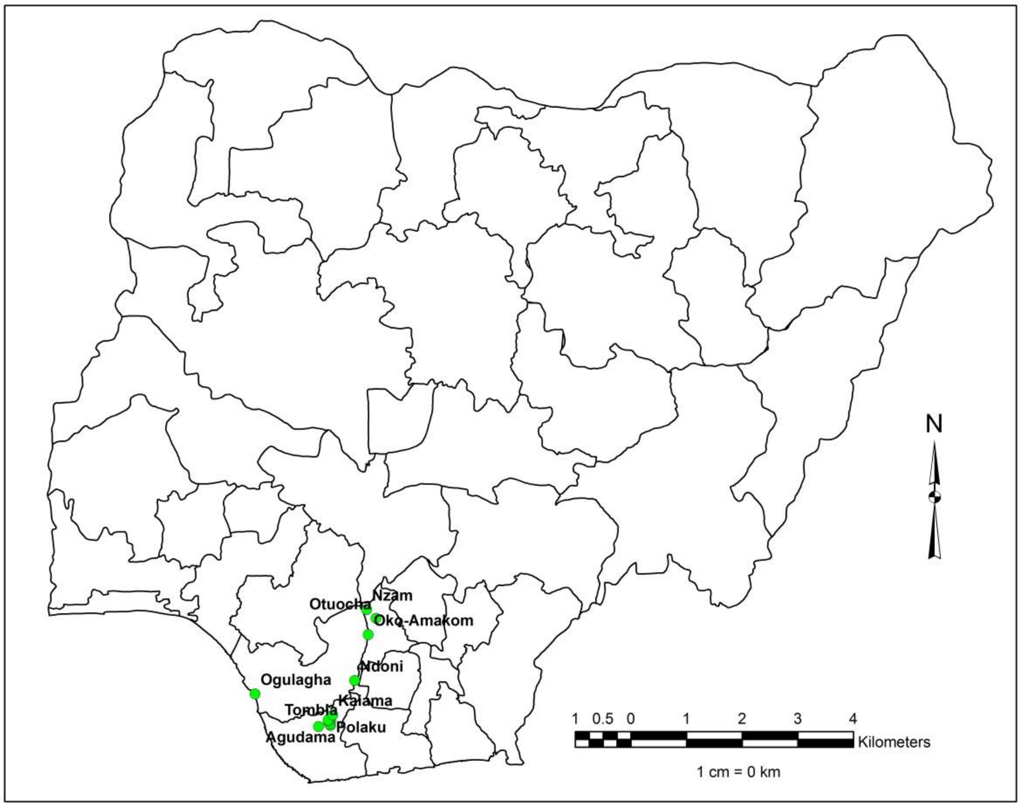


Figure 2: Map showing areas where Anambra Waxbills were sighted

Juveniles of Anambra Waxbill

Four juveniles of Anambra Waxbill were mistnettted during the survey at Agudama. The pictures taken were presented to Professor Manu (Director, APLORI), for identification and confirmation. The characteristics dun colour, red rump and patches of red pigment on the bill at the nares were considered means of identification.

Coker,2015

Plate 4: Juvenile Anambra Waxbill at Agudama farmland

Effects of Specific Vegetation Characteristics on the Abundance of Anambra Waxbill.

There was no significant effect of habitat parameters on the numbers of Anambara Waxbill sighted during the surveyed areas (see Table 2).

Table 2: Zero Altered Negative Binomial Model showing no significant effects of Specific Vegetation Characteristics on the Abundance of Anambra Waxbill across study sites

Count model coefficients (truncated negbin with log link):				
Variable	***Estimate***	***Std.Error***	***z-value***	***P***
(Intercept)	1.97	1.37	1.44	0.15
Grass height	0.00	0.01	-0.36	0.72
Grass with seeds	0.01	0.01	1.20	0.23
Ground cover (%)	-0.003	0.01	-0.22	0.83
Litter cover (%)	0.02	0.01	1.71	0.09
Number of big trees	-0.07	0.04	-1.58	0.11
Number of small trees	-0.0002	0.06	0.00	0.998
Number of stumps	0.01	0.13	0.06	0.95
Number of saplings	0.20	0.25	0.81	0.42
Number of fruiting trees	0.03	0.31	0.10	0.92
Number of flowering trees	-0.66	0.36	-1.85	0.06
Proportion of patch surveyed	-7.04	5.24	-1.34	0.18
Log(theta)	-0.85	0.55	-1.55	0.12

Effects of Specific Vegetation Characteristics on the Occurence of Anambra Waxbill.

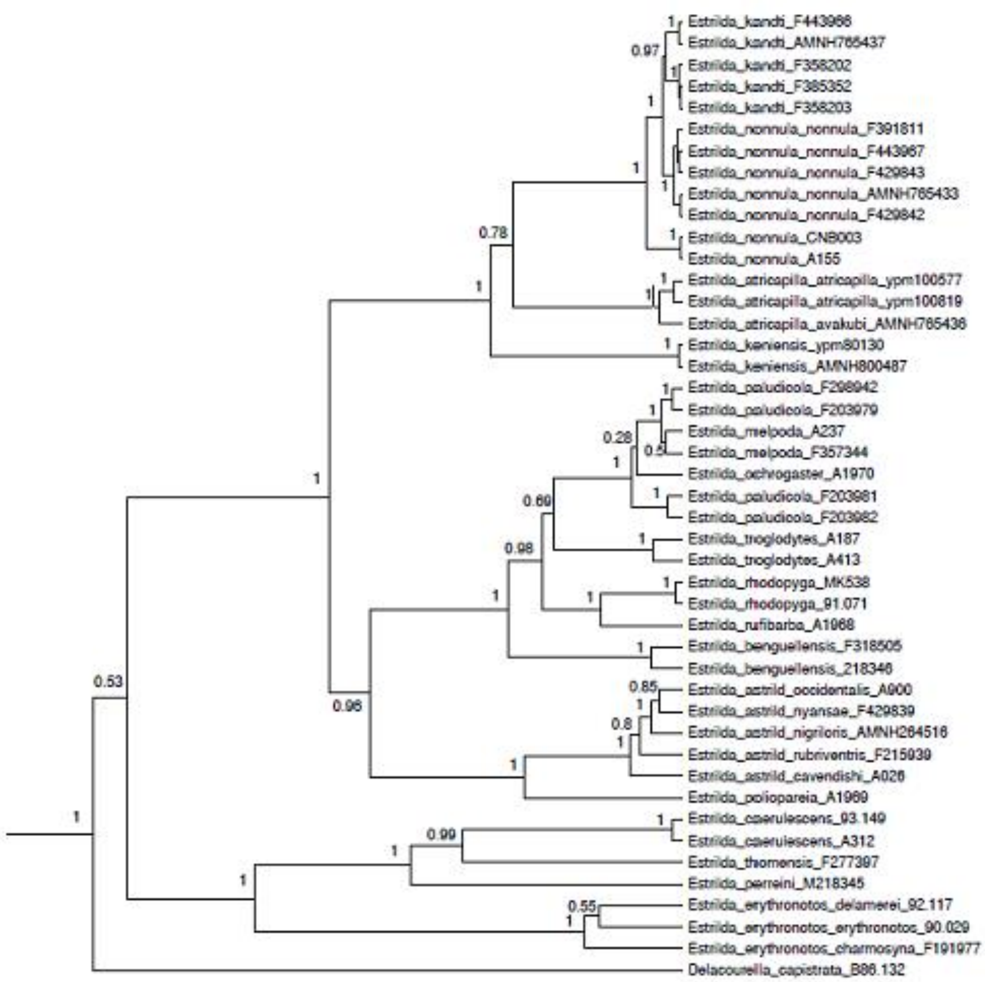
There were significant effects of some vegetation characteristics on the occurrence of Anambra Waxbill across study sites (Table 3). Anambra Waxbills are more likely to occur in areas with relatively taller grass, lower litter cover and in areas with more tree stumps: ZANB model - Grass height (B = 0.01, z = - 4.89, p <0.05), percentage litter cover (B = -0.02, z = - 2.39, p < 0.05), number of stump (B = 0.24, z = 2.32, p < 0.05).

Table 3: Zero Altered Negative Binomial Model showing the effects of habitat parameters on the distribution of Anambra Waxbill across study sites

Zero hurdle model coefficients (binomial with logit link):				
Variable	***Estimate***	***Std.Error***	***z-value***	***P***
(Intercept)	-3.05	0.63	-4.89	0.00
Grass height	**0.01**	**0.00**	**2.50**	**0.01**
Grass with seeds	0.01	0.01	1.01	0.31
Ground cover (%)	0.004	0.01	0.49	0.62
Litter cover (%)	**-0.02**	**0.01**	**-2.39**	**0.02**
Number of big trees	-0.02	0.03	-0.60	0.55
Number of small trees	0.05	0.04	1.07	0.29
Number of stumps	**0.24**	**0.10**	**2.32**	**0.02**
Number of saplings	-0.14	0.12	-1.15	0.25
Number of fruiting trees	-0.05	0.08	-0.68	0.50
Number of flowering trees	-0.02	0.15	-0.12	0.90
Proportion of patch surveyed	**13.03**	**2.28**	**5.71**	**0.00**

Phylogenetic Relationship between Anambra Waxbill, Fawn-breasted Waxbill and Arabian Waxbill

The Maximum Likelihood phylogenetic tree of Waxbill species reconstructed (see Figure 3) by Professor Michael Sorenson shows that with *Delacourella capistrata* as root the species of the phylogenetic tree among Waxbill species. This splits into two groups with 53% likelihood support level. Anambra Waxbill (*Estrilda poliopareia*) and Common Waxbill (*Estrilda astrild*) grouped into the same clade with 96% support level. However*, E. poliopareia* and *E. astrild* split into different species at 100% support level. The result also shows *E.poliopareia* as a species that evolved earlier than *E.rufibarba* and *E.paludicola*, which is contrary to previous classifications (Bannermann, 1949; Hall and Moreau, 1970; Clements, 2000; Sibley and Ahlquist, 1990; Serle, 1957).



Sorenson, 2015

Figure 3: The Phylogenetic tree showing the relationship of Anambra Waxbill to other Waxbills.

DISCUSSION

Effects of Specific Vegetation Characteristics on the Abundance of Anambra Waxbill

From the results obtained so far, the effects of habitat characteristics on the number of Anambra Waxbills sighted are not significant (Table 2). Anambra Waxbill could be taking advantage of all year-round availability of food sources because of almost year-round rainfall. Opportunistic feeding could also not be ruled out because of diversity of resources available especially different species of grass that could have different maturity periods. Anambra Waxbill being endemic to the area surveyed would have developed adaptations to the climate, vegetation composition and physiognomy of the area.

Effects of Grass Height on the Occurrence of Anambra Waxbill

Grass height has positive effects on the occurrence of Anambra Waxbill (Table 3). This positive effect (B=0.01, P,0.05) could be due to their feeding nature as granivores. They will easily access seeds on tall grasses, and probably they feed only on seeds of specific grass species that are characteristically tall, hence the taller the grass, the more it is possible that it is matured and bearing seeds that are available as resource to Anambra Waxbill. The choice of tall grasses could be of advantage behaviourally, allowing Anambra Waxbill both to forage and carry out vigilance for possible predators without additional energy cost. The grass height will also provide vantage points to attract mates, which could also suggest that the species is territorial. The swampy nature of the habitat will prove grass height to be beneficial as a '*landing'* and for building nests (Zufiaurre *et a*l., 2016; Smathers Libraries, 2013).

Grass height influences the habitat preferences of some bird species; for example, low grass height supports higher abundance of Kestrels, Thrushes and Starlings by providing opportunities for prey sighting, access to prey hiding and improved prey catchability in rice field; whereas higher grass height provide for camouflage and foraging opportunities for Grey Heron, *Ardea cinerea* and Meadow Pipit, *Anthus pratensis* (Whittingham and Devereux, 2008). Grass height influences nesting success in Greater Sandgrouse, *Pterocles burchelli* (Doherty *et al.*, 2014). Eurasian bitterns (*Botaurus stellaris*) prefer rice fields with taller and more vegetated plants (Longoni *et al.*, 2011).

Effects of percentage litter cover on the distribution of Anambra Waxbill

Although the percentage litter cover is statistically significant to the distribution of Anambra Waxbill (P <0.05), the parameter estimate of the model (B = - 0.02) implies a negative relationship (Table 3). Higher percentage litter cover though adds to the humus in the soil which could have positive effects for grass growth and productivity, but it will obstruct access to seed banks especially in patches with fewer grass stands. Hence, the more the percentage litter cover, the more it will deny Anambra Waxbill access to critical food source, impede efficient habitat utilization and possible colonization of potentially good habitat. BirdLife (2009) posited that though Anambra Waxbill is in a potentially good habitat, there are probable factors, yet unknown, hindering its wide distribution. Litter also provides fuel for wild fires, which could be adverse to species with small populations like Anambra Waxbill that could not afford such stochastic events (Scarff and Westoy, 2006). Leaf litter also attracts ants (Shatluck, 2000; Wang et al., 2001) which could attack birds' feet and plumage (Willis and Oniki, 1978). Reptiles attracted and camouflaged by leaf litter (Cates et al., 2002; Cogger, 2000) could predate on birds (British Columbia Wildlife Park, 2016). Therefore, avoidance of places with higher percentage litter cover will be important to continuing persistence of Anambra Waxbill.

Effects of number of stumps on the distribution of Anambra Waxbill

Numbers of stumps (Table 3) are indicators of loss of biomass, anthropogenic activities and despoliation of habitat. The impacts of increase in numbers of stumps (B=0.24, P<0.05) will be the elimination of stifling effects of big trees on the undergrowth vegetation including grasses that are important to Anambra Waxbill (Hikoshaka and Antern, 2012). Removal of tree stands will increase penetration of sunlight to plants that could have been in the understory. Opening of space will also give opportunities for dispersed grass seeds to colonize new places that could be beneficial resources available to Anambra Waxbill in the long run (Camprodon et al., 2008; Suwanrat et al., 2015). Although, this could be a tricky situation, the balance use of habitat resources will engender a situation whereby different taxa and guilds in a habitat, especially the Niger Delta, will carve their respective niches and thrive.

Effects of proportion of patch area surveyed

The proportion of patch area surveyed (B=13.03, P<0.05) was only included in the statistical analysis as a confounding variable in the model (Table 3). In any survey, while it may not be possible to cover every point, the field survey for Anambra Waxbill was particularly moderated by the peculiar topography of the area. In the course of selecting points randomly, gullies, quick sandbars, mudflats, swamps and deep waters were deliberately avoided. The topography of the Niger Delta and its estuaries, make the entire region a wetland that does not permit a continuous transect of considerable length. The lengths of the point transects used during this field study were considerably controlled by the patch size and the topography.

Generally, a lack of significant effect of habitat parameters on the abundance of Anambra Waxbill may be indicative of a species that is below carrying capacity and with a lot of suitable habitats and resources to go round so that there is not any obvious aggregation in habitats with specific characteristics. But, the significant effect of these habitat parameters on occurrence (distribution) seems reasonable because as 0 and 1 data, it clearly highlights the habitat characteristics that are likely to determine whether they will use the habitats or not, but not necessarily if they aggregate there or not. Ribeiro and Fernandes (2000) had suggested that multiple factors drive the gradual decline of narrow endemic species' population size and density; while Chan et al (2010) posited that specific ecological and anthropogenic factors changing the frequency of extremely small populations differ among species. Hence, resource availability (Merinero et al., 2014), recruitment (Smith et al., 2014), genetic variability (Isik, 2011), historical and evolutionary processes (Willis, 1922; Lesica et al., 2006) contribute to the ability of an endemic species to multiply and be widely distributed. Although, Gibson et al. (2008) posited that endemic species with small population size, high isolation and highly disjunct distribution will experience rarity, yet a study of genetic diversity between and within populations will be required to give insights into its population stability.

Thus, depending on species-specific relationship between a species abundance, distribution and its interactions with habitat parameters ; a species may be locally rare in most places but, abundant in few places (Hanse et al., 2013, Buckland et al., 2014).

CONCLUSION AND FUTURE DIRECTION

Baseline data on abundance of Anambra Waxbill has been collected in Nigeria, as all historical and reported sites were visited. I have sighted about 500 individuals so far in Nigeria. The discovery of new populations at Oko-Amakom and Ogulagha portend great potentials for the species, as it was thought to be limited to the Ekpetiama areas of Bayelsa State in Nigeria (Roux and Otobotekere, 2005). Oko-Amakom is contiguous to Onitsha and Aguleri; this discovery is the most recent record of the species after more than 60 years. This result also suggests wider distribution of the species than was previously reported. This should be explored further as suggested by Roux and Otobotekere (2005).

Juveniles of Anambra Waxbill were recorded for the first time during this survey (see Plate 4). This opens opprtunities for a detailed study of the breeding biology of the species. Birdlife (2013) had pointed out the paucity of data in respect of the breeding biology and other aspects of the species natural history.

Non-sighting of the species at the historical sites namely Badagry and Topo Island after repeated visits might point to local extinction because of socio-economic pressures causing rapid loss of habitat to urbanisation, development of infrastructure and heavy industries.

The effects of vegetation characteristics on the abundance of Anambra Waxbill in Nigeria was not found to be significant, whereas, the specific vegetation characteristics that better support wider distribution of Anambra Waxbill are now known.

More fieldwork is required, to further understand the factors underpinning the persistence of the species predictable by vegetation characteristics; likewise the effects of seasonal variation on abundance and distribution of Anambra Waxbill.

The population of Anambra Waxbill reported for Benin Republic needs to be ground – truthed. Together with the collection of blood and feather samples for molecular analysis that will help to establish the possibility of gene flow between the populations in Nigeria and the ones in Benin Republic. The data and results therefrom will become vital in delineating source and sink populations.

Some blood and feather samples have been collected from the populations in Nigeria. More samples are required so that the taxonomic status can be resolved and provide answers to the pending questions on the degree of relatedness within and between populations.

The laboratory analysis of feathers already collected will also be useful to determine the level of heavy metal contaminations. This is a mean of measuring the impacts of anthropogenic activities on the persistence of Anambra Waxbill, as it relates to oil exploration which is a major economic activity in the Niger delta region.

The level of awareness about Anambra Waxbill and its conservation status is very low in the study sites visited. Of the host communities visited, it was at Tombia that there was a little awareness of the species by the local Chief. It will take sustained advocacy and public enlightenment in these host communities to promote conservation actions and community ownership of the species.

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