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Diversity and distribution of vascular macrophytes in Ansupa Lake, Odisha, India

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Abstract. Panda M, Samal RN, Bhatta KS, Lenka S, Rout J, Patra HK, Nanda S. 2018. Diversity and distribution of vascular macrophytes in Ansupa Lake, Odisha, India. Bonorowo Wetlands 1: 1-12. Macrophytes are indispensable component of any wetlands. They are the base of the trophic structure and variously affect function of aquatic ecosystem. Large invasion of macrophytes enforced for present studies in Ansupa Lake, the largest freshwater lake of the state Odisha (India) to identify the causative plant species. Regular field inspection, quadratic sampling and specimen collections were carried to identify the present macrophytes of the lake and their quantitative aspects like frequency of occurrences, abundance, values of diversity indices, adaptation and growth forms and species distribution etc. A total of 244 macrophyte species were identified that includes 182 semi-aquatic and 62 obligatory aquatic macrophytes. The latter group had 35% submerged, 15% free floating, 31% rooted floating and 19% marshy plant species. The comparison of growth form showed 66% annuals and remaining 34% perennial plants. The diversity indices resulted, Simpson complement index-0.561, Shannon-Weiner index-1.367, Species richness index 3.079 and Species evenness index-0.156. The study showed that the lake provides suitable habitats for existence of a diverse group of macrophytes but still due to large invasion of few species has threatened the lake which needs to be managed properly to restore the health of this natural resource for the benefit of mankind.

Keywords: Ansupa Lake, conservation, macrophyte diversity, species invasion

INTRODUCTION

Wetlands are the hotspots of biological diversity and invaluable for sustainable living. Plants in water are called macrophytes (Dodds 2002). They act as “biological engineers” in restoring water quality (Byers et al. 2006). It includes both flowering and non-flowering plants that start their life in and around water bodies (Chambers et al. 2008). A total of 2614 aquatic vascular macrophytes occur globally which represent only 1% of the total number of vascular plants (Ansari et al. 2017). Total number of aquatic plant species in Indian freshwaters exceeds 1200 (Gopal 1995). Many species of aquatic plant are invasive species (Oyedeji and Abowei 2012). These plants cause local losses of species diversity and alter ecosystem structure, resulting in a significant negative impact on aquatic biodiversity and water quality (Brundu 2015; Chamier et al. 2012; Wang et al. 2016; Zedler and Kercher 2004). In India, over 140 aquatic plants are reported to have attained the status of aquatic weeds in different situations (Gupta 2012; Naskar 1990; Shah and Reshi 2012; Varshney et al. 2008).

Ansupa Lake, the present study sites is the largest fresh water lake of the state Odisha (India) (Mohanty and Das 2008) and a lake of national importance (Das and Mohanty 2008). The lake provides livelihood provisions like fishing i.e., small indigenous fishes, table size fishes and ornamental fishes; agriculture, i.e., rice cultivation; edible aquatic plants and ecotourism due to its unique biodiversity and natural scenery (Sarkar et al. 2015). More than 25,000 fishermen and local residence make their livelihood on the lake water (Das and Mohanty 2008; Mohanty and Das 2008). The average water depth of the lake was 4 meters (Das and Mohanty 2008). The lake receives annual rainfall between 800mm to 1300mm (Das and Mohanty 2008; Panda et al. 2016) and most during months of July and August, each year. It hosts 44 species of phytoplankton, 32 species of zooplanktons and 30 species of fishes (Patra and Patra 2007). Panda et al. (2016) for the first time reported occurrence of Hygroryza aristata (Retz.) Nees. ex Wt. and Arn., a wild relative of edible rice in Ansupa Lake as the only habitat in the state for this species. There is few published work on Ansupa Lake and the macrophytes study is very poorly reported (Das and Mohanty 2008; Mohanty and Das 2008; Varshney et al. 2008; Sarkar et al. 2015; Panda et al. 2016). All previous studies reported the progressive degradation conditions of the lake due to siltation, shrinkage of water spread area and invasions of aquatic plants (Das and Mohanty 2008; Mohanty and Das 2008; Sarkar et al. 2015; Panda et al. 2016). Knowing the importance of Ansupa Lake, present studies were designed to identify the macrophyte diversity, the problematic weeds that need to be managed properly for the long term conservation of indigenous biota and creation of better livelihood opportunity from the lake.
MATERIALS AND METHODS

Study area
Ansupa Lake is the largest fresh water lake of Odisha State, India, situated between latitude 20° 26' 21'' to 20° 28' 52'' N and 85° 36' 25'' to 85° 36' 0'' E longitude on the river bank of Mahanadi (Figure 1). The area of the lake is around 375 acres and 385 acres during the dry and rainy seasons, respectively (Mohanty and Das 2008).

Field data collection and floristic study
The floristic studies were carried during November 2014 and an extensive regular field work from April to November 2017. The recorded macrophytes were identified with the help of available both regional and international scientific literatures (Calvert and Liessmann 2014; Campbell et al. 2010; Crow and Hellquist 2000; Das 2012; Gerber et al. 2004; Ghosh 2005; Gupta 2012; Haines 1921-1925; Naskar 1990). The scientific name and author citation were checked with, The plant list (http://www.theplantlist.org/) and International Plant Names Index (http://www.ipni.org/ipni/plantnamesearchpage.do). Quantitative status and ecological parameters were calculated from 25 fixed random plots, i.e. size, 1m × 1m (Figure 1).

Data analysis
The quadratic parameters like, Frequency and Abundance (Upadhyay et al. 2009), Whitford’s index (A/F) (Whitford 1949), Species richness index (Margalef 1958), Simpson complement index (1-Ds) from Simpson Dominance index (Simpson 1949), Shannon-Wiener index (Shannon and Wiener 1963) and Species evenness index (J) (Pielou 1975) were calculated as follows:

- Frequency = $\frac{\text{No. of plots in which a species occurs}}{\text{Total no of plots sampled}} \times 100$
- Abundance = $\frac{\text{Total number of Individuals of a species in all quadrats}}{\text{Number of quadrats in which the species occurred}}$
- Species dispersion or Whitford’s index ($\frac{A}{F}$) = $\frac{\text{Abundance}}{\text{Frequency}}$
- Species richness index (RI) = $\frac{S - 1}{\ln N}$ as per Margalef (1958)

Where, S is the total number of species in the community and N is the total number of individuals of all species of a community.

Figure 1. Location map of Ansupa Lake, Cuttack District, Odisha, India
RESULTS AND DISCUSSION

A total of 244 vascular macrophytes were identified to occur in and shoreline areas of the lake. Out of the total record, 238 species were of flowering plants, i.e., Angiosperms (Table 1) and 6 species of non-flowering macrophytes, i.e., Pteridophyte (Table 2). All six pteridophytes were strictly aquatic species; they belong to only two families (i.e., Marsileaceae and Salviniaceae) and except Azolla microphylla Kaulf., which was an annual species others were perennial in their growth form (Table 2). The angiospermic macrophytes belong to a total of sixty families. Among these families, Poaceae and Cyperaceae were recorded as the most diversified families (Figure 2).

The classification of total angiosperms revealed 137 (58%) dicot species and 101 (42%) monocot species (Figure 4). Among the dicot group, only 26 (19%) species were strictly aquatic and 111 (81%) species were semi-aquatic plants (Figure 5). Similarly, the monocot group had 30 species (30%) and 71 species (70%) as aquatic and semi-aquatic plants, respectively (Figure 6). The comparison of growth form showed 160 species (66%) annual and remaining 84 species (34%) as perennial macrophytes (Figure 7). The classification of total aquatic species displayed 35% submerged, 15% free floating, 31% rooted floating and 19% marshy plant species (Figure 8).

The study found occurrence of wide habitat variability that helped establishment of different group of aquatic and semi-aquatic vascular macrophytes in the lake. Many macrophytes showed seasonal changes of population status, influenced by water level (Dalu et al. 2012). This affects the value of diversity index of the ecosystem, as calculated by ratio between the number of species and the number of individuals in that community (Ansari et al. 2017). The low value of species evenness index showed the present species were not equally abundant, some species dominated over others. The lake hosts some unique macrophytes that found rarely elsewhere in the state. Hygroryza aristata (Retz.) Nees, Ex Wt. & Arn. and Oryza rufipogon Griff., the wild
Figure 3. Classification as per habitat requirement: Aquatic and semi-aquatic plants (%) 

Figure 6. Classification of monocots into habitat groups: Aquatic and semi-aquatic monocots (%) 

Figure 4. Classification into Angiosperm group: Diversity of dicot and monocot species (%) 

Figure 7. Classification of macrophytes into growth forms: Growth form of macrophytes (%) 

Figure 5. Classification of dicots into habitat group: Aquatic and semi-aquatic dicots (%) 

Figure 8. Classification of aquatic plants into their adaptation group: Adaptation forms of aquatic plants (%) 

Figure 9. Diversity indices from quadrate data

Relative of edible rice were a common occurrence in the lake (Plate 1). The aesthetically important and endangered plant species, *Gloriosa superba* L. has been recorded from shoreline areas of the lake for the first time (Plate 1). The semi-aquatic plants were diverse and many showed seasonal growth. Many of them were small herbaceous annual plants.

Strong infestation of *Nelumbo nucifera* Gaertn., *Eichhornia crassipes* (Mart.) Solm-Laub., *Salvinia molesta* D. S. Mitch, *Ceratophyllum demersum* L., *Hydrilla verticillata* (L.f.) Royle, *Najas indica* (Willd) Cham.; *Hymenachne amplexicaulis* (Rudge) Nees, other grasses and marshy vegetation were found negatively affecting the lake (Plate 2). Soil erosion from surrounded hills and siltation, decreased water flow due to closing of inlets and outlets with Mahanadi River, intensive fertilizer load are the possible factors for degradation of the lake.
Table 1. List of Angiospermic macrophyte recorded from Ansupa Lake, Odisha, India

| Plant family | Si. No. | Plant species | Plant group | Macrophyte type | Life form |
|--------------|---------|---------------|-------------|-----------------|----------|
| Acanthaceae  | 1       | Andrographis paniculata (Burm.f.) Wall. ex Nees | D           | Semi-aquatic    | Annual   |
|              | 2       | Hygrophila auriculata (Schum) Heine             | D           | Semi-aquatic    | Annual   |
|              | 3       | Hygrophila schulli (Buch.-Ham.) M.R.Almeida & S.M. Almeida | D        | Semi-aquatic    | Annual   |
|              | 4       | Justicia diffusa Willd.                        | D           | Semi-aquatic    | Annual   |
|              | 5       | *Ruellia tuberosa L.                           | D           | Semi-aquatic    | Annual   |
| Aizoaceae    | 6       | *TriandHEMA portulacastrum L.                  | D           | Semi-aquatic    | Annual   |
| Alismataceae | 7       | *Alisha plantago-aquatica L.                   | M           | Aquatic (S)     | Annual   |
|              | 8       | Limnophyton obtusifolium (L.) Miq.            | M           | Aquatic (S)     | Annual   |
|              | 9       | Sagittaria sagittifolia L.                     | M           | Aquatic (S)     | Annual   |
|              | 10      | Sagittaria guayanensis var. lappula D. Don     | M           | Aquatic (S)     | Annual   |
| Amaranthaceae| 11      | Sagittaria trifolia L.                         | M           | Aquatic (S)     | Annual   |
|              | 12      | *Achyranthes aspera L.                         | D           | Semi-aquatic    | Annual   |
|              | 13      | Aerva lanata (L.) Juss. ex Schult.            | D           | Semi-aquatic    | Annual   |
|              | 14      | *Alternanthera paronymchioides A. St-Hil.      | D           | Semi-aquatic    | Annual   |
|              | 15      | *Alternanthera philoxeroides (Mart.) Griseb.   | D           | Semi-aquatic    | Annual   |
|              | 16      | *Alternanthera sessilis (L.) DC.               | D           | Semi-aquatic    | Annual   |
|              | 17      | *Amaranthus spinosus L.                        | D           | Semi-aquatic    | Annual   |
|              | 18      | *Amaranthus viridis L.                         | D           | Semi-aquatic    | Annual   |
|              | 19      | *Celosia argentea L.                          | D           | Semi-aquatic    | Annual   |
|              | 20      | *Gomphrena celosioides Mart.                  | D           | Semi-aquatic    | Annual   |
| Amaryllidaceae| 21      | Crinum latifolium L.                           | M           | Aquatic (S)     | Annual   |
|              | 22      | Crinum viviparum (Lam.) R.Ansari & V.J.Nair   | M           | Aquatic (RF)    | Annual   |
| Apiaceae     | 23      | Centella asiatica (L.) Urb.                   | D           | Semi-aquatic    | Perennial |
|              | 24      | *Hydrocotyle modesta Cham. & Schltld.         | D           | Semi-aquatic    | Perennial |
| Aponogetonaceae| 25     | Aponogeton natans (L.) Engl. & Krause         | M           | Aquatic (S)     | Annual   |
| Araceae      | 26      | Alocasia indica (Roxb.) Schott                | M           | Semi-aquatic    | Perennial |
|              | 27      | Colocasia esculenta (L.) Schott               | M           | Semi-aquatic    | Perennial |
|              | 28      | *Pistia stratiotes L.                         | M           | Aquatic (FF)    | Perennial |
| Asteraceae   | 29      | *Ageratum conyzoides L.                       | D           | Semi-aquatic    | Perennial |
|              | 30      | Blumea lacera (Burm.f.) DC.                   | D           | Semi-aquatic    | Annual   |
|              | 31      | Caesalia asillaris Roxb.                      | D           | Semi-aquatic    | Annual   |
|              | 32      | *Chromolaena odorata (L.) King & H.E. Robins. | D           | Semi-aquatic    | Perennial |
|              | 33      | Cyanthillium cinereum (L.) H. Rob             | D           | Semi-aquatic    | Annual   |
|              | 34      | *Eclipta alba (L.)                            | D           | Semi-aquatic    | Annual   |
|              | 35      | Eclipta prostrata (L.) L.                     | D           | Semi-aquatic    | Annual   |
|              | 36      | Enydra fluctuans Lour.                        | D           | Aquatic (S)     | Annual   |
|              | 37      | Emilia sonchifolia (L.) DC                    | D           | Semi-aquatic    | Annual   |
|              | 38      | *Gnaphaliun polycaulon Pers.                  | D           | Semi-aquatic    | Annual   |
|              | 39      | Grangea maderaspatana L.                      | D           | Semi-aquatic    | Annual   |
|              | 40      | *Mikania cordata (Burm.f.) Robinson           | D           | Semi-aquatic    | Annual   |
|              | 41      | Sphaeranthus indicus L.                       | D           | Semi-aquatic    | Annual   |
|              | 42      | Spilanthes paniculata Wall. Ex DC.            | D           | Semi-aquatic    | Annual   |
|              | 43      | *Syndrerella nodiflora (L.) Gaertn.           | D           | Semi-aquatic    | Annual   |
|              | 44      | *Xanthium strumarium L.                       | D           | Semi-aquatic    | Annual   |
| Boraginaceae | 45      | Coldenia procumbens L.                        | D           | Semi-aquatic    | Annual   |
|              | 46      | Heliotropium indicum L.                       | D           | Semi-aquatic    | Annual   |
| Capparaceae  | 47      | Cleome monophylla L.                          | D           | Semi-aquatic    | Annual   |
|              | 48      | Cleome viscosa L.                             | D           | Semi-aquatic    | Annual   |
| Cariophyaceae| 49      | *Polycarpon prostratum (Forssk,) Asc. & Sch.  | D           | Semi-aquatic    | Annual   |
| Ceratophyllaceae| 50  | Ceratophyllum demersum L.                      | D           | Aquatic (S)     | Perennial |
| Colchicaceae | 51      | Gloriosa superba L.                           | M           | Semi-aquatic    | Perennial |
| Commelinaceae| 52      | Commelina benghalensis L.                     | M           | Semi-aquatic    | Perennial |
|              | 53      | Commelina erecta L.                           | M           | Semi-aquatic    | Perennial |
|              | 54      | Commelina longifolia Lam.                     | M           | Semi-aquatic    | Perennial |
|              | 55      | Cyanosis axillaris (L.) D Don ex Sweet        | M           | Semi-aquatic    | Perennial |
|              | 56      | *Evolvulus nummularius (L.)                   | M           | Semi-aquatic    | Perennial |
|              | 57      | Murdannia nudiflora (Linn.) Brenan.           | M           | Semi-aquatic    | Annual   |
|              | 58      | Murdannia spirata (L.) Bruckn.                | M           | Semi-aquatic    | Annual   |
| Convolvulaceae| 59     | *Ipomoea aquatica Forssk.                    | D           | Aquatic (RF)    | Perennial |
|              | 60      | *Ipomoea carnea Jacq. ssp. Fistulos (Mart. ex Choisy) Austin | D     | Semi-aquatic    | Perennial |
| No. | Species                                                                 | Life Form | Growth Habit |
|-----|------------------------------------------------------------------------|-----------|--------------|
| 61  | *Ipomoea pes-tigridis* L.                                               | D         | Semi-aquatic Perennial |
| 62  | Merremia tridentata (L.) Hall. f.                                       | D         | Semi-aquatic Perennial |
| 63  | Costus speciosus (J. Koenig) Sm.                                        | M         | Semi-aquatic Perennial |
| 64  | Bryophyllum calycinum Salisb.                                           | D         | Semi-aquatic Perennial |
| 65  | Muka maderaspatana (L.) M. Roem.                                        | D         | Semi-aquatic Annual  |
| 66  | Cucumis melo L.                                                         | D         | Semi-aquatic Annual  |
| 67  | Cyperus alopecuroides Roth.                                             | M         | Semi-aquatic Annual  |
| 68  | *Cyperus brevifolius* (Roott.) Hassk.                                   | M         | Semi-aquatic Perennial |
| 69  | Cyperus cephalotes Vahl.                                                | M         | Semi-aquatic Perennial |
| 70  | Cyperus compressus L.                                                   | M         | Semi-aquatic Annual  |
| 71  | Cyperus corymbosus Roth.                                                | M         | Semi-aquatic Perennial |
| 72  | Cyperus diiformis L.                                                    | M         | Semi-aquatic Annual  |
| 73  | Cyperus haspan L.                                                       | M         | Semi-aquatic Annual  |
| 74  | Cyperus imbricatus Retz.                                                | M         | Semi-aquatic Perennial |
| 75  | Cyperus iria L.                                                         | M         | Semi-aquatic Annual  |
| 76  | Cyperus platystylis R. Br.                                              | M         | Semi-aquatic Perennial |
| 77  | Cyperus polystachyos Roth.                                              | M         | Semi-aquatic Perennial |
| 78  | Cyperus rotundus L.                                                     | M         | Semi-aquatic Perennial |
| 79  | *Cyperus strictosus* L.                                                 | M         | Semi-aquatic Perennial |
| 80  | Eleocharis acutangula (Roxb.) schutt.                                   | M         | Aquatic (RE) Perennial |
| 81  | Echinocloa crus-galli (L.) P. Beauv.                                    | M         | Semi-aquatic Annual  |
| 82  | Eleocharis dulcis (Burmf.) Trin. ex Henschel                            | M         | Semi-aquatic Perennial |
| 83  | Fimbristylis dipsacea (Roott.) C.B. Clarke                              | M         | Semi-aquatic Annual  |
| 84  | Fimbristylis ferruginea (L.) Vahl.                                      | M         | Semi-aquatic Perennial |
| 85  | Fimbristylis littoralis Gaudich.                                        | M         | Semi-aquatic Annual  |
| 86  | Fimbristylis milacea (L.) Vahl.                                         | M         | Semi-aquatic Annual  |
| 87  | Fuirena ciliaris (L.) Roxb.                                             | M         | Semi-aquatic Annual  |
| 88  | *Kyllinga tenuifolia* Stud.                                             | M         | Semi-aquatic Annual  |
| 89  | Lipocarpa chinensis (Osbeck) J. Kern.                                   | M         | Semi-aquatic Annual  |
| 90  | Cyperous compactus Retz.                                                | M         | Semi-aquatic Annual  |
| 91  | Pycreus pumilus (L.) Nees                                               | M         | Semi-aquatic Annual  |
| 92  | Schoenoplectus articularus (L.) Palla                                   | M         | Semi-aquatic Annual  |
| 93  | Schoenoplectus grossus (L.) Palla                                       | M         | Semi-aquatic Perennial |
| 94  | Schoenoplectella supina (L.) Lye                                        | M         | Semi-aquatic Annual  |
| 95  | *Bergia ammannioides* Roxb. ex Roth.                                    | D         | Semi-aquatic Annual  |
| 96  | Bergia capensis L.                                                      | D         | Semi-aquatic Perennial |
| 97  | Eriocaulon quinquangulare L.                                            | M         | Semi-aquatic Perennial |
| 98  | Acalypa indica L.                                                       | D         | Semi-aquatic Annual  |
| 99  | *Croton bomplandianus* (Baill.) Kuntze                                  | D         | Semi-aquatic Annual  |
| 100 | Euphorbia hirta L.                                                      | D         | Semi-aquatic Annual  |
| 101 | *Euphorbia prostrata* Aiton.                                            | D         | Semi-aquatic Annual  |
| 102 | Jatropha gossypifoila L.                                                | D         | Semi-aquatic Annual  |
| 103 | *Phyllanthus tenellus* Roxb.                                            | D         | Semi-aquatic Annual  |
| 104 | *Ricinus communis* L.                                                   | D         | Semi-aquatic Perennial |
| 105 | Aescynonomene aspera L.                                                | D         | Semi-aquatic Annual  |
| 106 | Aescynonomene indica L.                                                | D         | Semi-aquatic Annual  |
| 107 | Alysicarpus vaginalis (L.) DC.                                          | D         | Semi-aquatic Annual  |
| 108 | *Cassia tora* L.                                                        | D         | Semi-aquatic Annual  |
| 109 | *Crotalaria pallida* Aiton                                              | D         | Semi-aquatic Perennial |
| 110 | Crotalaria quinquefolia L.                                              | D         | Semi-aquatic Perennial |
| 111 | Zornia diphylla (L.) Pers.                                              | D         | Semi-aquatic Annual  |
| 112 | Senna obtusifolia (L.) H.S. Irwin. & Barneby                             | D         | Semi-aquatic Annual  |
| 113 | *Senna occidentalis* (L.) Link                                         | D         | Semi-aquatic Annual  |
| 114 | Sesanbia hispina (Jacc.) W.F. Wt.                                      | D         | Semi-aquatic Annual  |
| 115 | Hoppea dichotoma Wild.                                                 | D         | Semi-aquatic Annual  |
| 116 | Blyxa echinosperma (Clarke) Hook.f.                                     | M         | Aquatic (S) Annual  |
| 117 | Hydriella verticillata (L.f.) Royle                                     | M         | Aquatic (S) Perennial |
| 118 | Nechamandra alternifolia (Roxb. ex Wight) Thw.                         | M         | Aquatic (S) Perennial |
| 119 | Ottelia alismoides (L.) Pers.                                           | M         | Aquatic (S) Perennial |
| 120 | Vallisneria natans (Lour.) H. Har.                                      | M         | Aquatic (S) Annual  |
| 121 | Hydroea cymalica (L.) Vahl.                                             | D         | Aquatic (RE) Annual  |
| 122 | Anisomeles indica (L.) O. Kuntze.                                       | D         | Semi-aquatic Perennial |
| 123 | Leucas aspera (Willd.) Link                                             | D         | Semi-aquatic Annual  |
| 124 | Pogostemon quadripilis (Benth.) F. Muell.                               | D         | Semi-aquatic Annual  |
| 125 | *Spirodela polyrhiza* (L.) Schleid.                                     | M         | Aquatic (FF) Perennial |
| 126 | Lemma gibba L.                                                          | M         | Aquatic (FF) Annual  |
| 127 | Lemma aquinotchialis Welw | M | Aquatic (FF) | Annual |
| 128 | Wolffia globosa (Roxb.) Hartog & Vander Plas | M | Aquatic (FF) | Annual |
| 129 | Utricularia austral Lour. | D | Aquatic (S) | Annual |
| 130 | Utricularia inflata Forssk. | D | Aquatic (S) | Annual |
| 131 | Utricularia bifida L. | D | Aquatic (S) | Annual |
| 132 | Lindernia crustacea (L.) F.Muell. | D | Semi-aquatic | Annual |
| 133 | Ammamia bicacca L. | D | Semi-aquatic | Annual |
| 134 | Ammamia multiflora Roxb. | D | Semi-aquatic | Annual |
| 135 | Ammamia octandra L.f. | D | Semi-aquatic | Annual |
| 136 | Rotala densiflora (Roth. ex Roem. & Schult.) Koehne | D | Semi-aquatic | Annual |
| 137 | Rotala indica (Willd.) Koehne | D | Semi-aquatic | Annual |
| 138 | Abutilon indicum (L.) Sweet | D | Semi-aquatic | Annual |
| 139 | Corchorus aetouans L. | D | Semi-aquatic | Annual |
| 140 | Sida cordifolia L. | D | Semi-aquatic | Annual |
| 141 | Urena lobata L. | D | Semi-aquatic | Annual |
| 142 | 'Martynia annua L. | D | Semi-aquatic | Annual |
| 143 | Nympoiodes hydrophylia (Lour.) Kuntze | D | Aquatic (RF) | Annual |
| 144 | Nympoiodes indica (L.) Kuntze | D | Aquatic (RF) | Annual |
| 145 | 'Mimosa pudica L. | D | Semi-aquatic | Perennial |
| 146 | Neptunia oleracea Lour. | D | Aquatic (RF) | Perennial |
| 147 | Neptunia plena (L.) Benth. | D | Aquatic (RF) | Perennial |
| 148 | Glinus oppositifolius (L.) Aug. DC | D | Semi-aquatic | Annual |
| 149 | Mollugo pentaphylla L. | D | Semi-aquatic | Annual |
| 150 | Myriophyllum tetrandrum Roxb. | D | Aquatic (RE) | Annual |
| 151 | *Myriophyllum aquaticum (Vell.) Verdc. | D | Aquatic (RE) | Perennial |
| 152 | Myriophyllum verticillatum L. | D | Aquatic (RE) | Annual |
| 153 | Najas faveolata A. Br. ex Magam. | M | Aquatic (S) | Perennial |
| 154 | Najas indica (Wild) Cham. | M | Aquatic (S) | Perennial |
| 155 | Najas marina L. | M | Aquatic (S) | Perennial |
| 156 | Nolmbo nucifera Gaertn. | D | Aquatic (RF) | Perennial |
| 157 | Nyctaginaceae | D | Semi-aquatic | Annual |
| 158 | Boerhavia diffusa L. | D | Semi-aquatic | Annual |
| 159 | Boerhavia repens L. | D | Semi-aquatic | Annual |
| 160 | Euryale ferox Salisb. | D | Aquatic (RF) | Perennial |
| 161 | Nymphaea nouchali Burm.f. | D | Aquatic (RF) | Perennial |
| 162 | Nymphaea pubescens Willd. | D | Aquatic (RF) | Perennial |
| 163 | Nymphaea rubra Roxb. ex Andrews | D | Aquatic (RF) | Perennial |
| 164 | Ludwigia prostrata Roxb. | D | Semi-aquatic | Annual |
| 165 | Ludwigia adscendens (L.) H. Harra | D | Aquatic (RF) | Perennial |
| 166 | Ludwigia octovalvis (Jacq.) P.H. Raven | D | Semi-aquatic | Annual |
| 167 | Ludwigia perennis L. | D | Semi-aquatic | Annual |
| 168 | Oxalis corniculata L. | D | Semi-aquatic | Annual |
| 169 | Plantaginaceae | D | Semi-aquatic | Annual |
| 170 | *Scoparia dulcis L. | D | Semi-aquatic | Annual |
| 171 | Apulada mutica L. | M | Semi-aquatic | Annual |
| 172 | Arundinella pumila (Hochst. ex A.Rich) Steud | M | Semi-aquatic | Annual |
| 173 | Axonopus compressus (Sw.) P.Beauv. | M | Semi-aquatic | Perennial |
| 174 | Brachiaria deflexa (Schumach.) C.E.Hubb. ex Robyns | M | Semi-aquatic | Annual |
| 175 | Brachiaria mutica (Forssk.) Stapf. | M | Semi-aquatic | Perennial |
| 176 | Brachiaria ramosa (L.) Stapf | M | Semi-aquatic | Annual |
| 177 | Brachytria leptans (L.) C.A.Gardner & C.E.Hubb | M | Semi-aquatic | Annual |
| 178 | *Chloris barbata Sw. | M | Semi-aquatic | Annual |
| 179 | Cyrtococcum longipes (Hook.f.) A.Camus | M | Semi-aquatic | Perennial |
| 180 | Cydonon dactylon (L.) Pers. | M | Semi-aquatic | Perennial |
| 181 | Dichanthelium sp. | M | Semi-aquatic | Annual |
| 182 | Echinochloa colona (L.) Link | M | Semi-aquatic | Annual |
| 183 | Echinochloa crus-galli (L.) P.Beauv. | M | Semi-aquatic | Annual |
| 184 | Echinochloa stagnina (Retz.) Beauv. | M | Semi-aquatic | Annual |
| 185 | Eleusine indica (L.) Gaertn | M | Semi-aquatic | Annual |
| 186 | Elytrophorus spicatus (Wild.) A. Camus | M | Semi-aquatic | Annual |
| 187 | Eragrostis cilianis (L.) R.Br. | M | Semi-aquatic | Annual |
| 188 | Eragrostis gangetica (Roxb.) Steudel | M | Semi-aquatic | Annual |
| 189 | Eragrostis japonica (Thunb.) Trin. | M | Semi-aquatic | Perennial |
| 190 | Eragrostis pilosa (L.) P.Beauv. | M | Semi-aquatic | Annual |
| 191 | Eragrostis tenella (L.) P.Beauv.ex Roem. & Schult. | M | Aquatic (RF) | Perennial |
| 192 | *Hymenachne amplexicaulis (Rudge) Nees | M | Aquatic (RF) | Perennial |
| 193 | Leersia hexandra Sw. | M | Semi-aquatic | Perennial |
Table 2. List of Non-flowering (Pteridophyte) macrophytes of Ansupa Lake (Odisha), India

| Family         | S. No. | Plant species          | Habitat group | Life form |
|----------------|--------|------------------------|---------------|-----------|
| Marsileaceae   | 1      | Marsilea minuta L.     | Aquatic (RF)  | Perennial |
|                | 2      | Marsilea quadrifolia L.| Aquatic (RF)  | Perennial |
| Salviniaeae    | 3      | *Azolla microphylla*   | Aquatic (FF)  | Annual    |
|                | 4      | *Azolla pinnata*       | Aquatic (FF)  | Perennial |
|                | 5      | *Salvinia minima*      | Aquatic (FF)  | Perennial |
|                | 6      | *Salvinia molesta*     | Aquatic (FF)  | Perennial |

Note: RF=Rooted floating, FF=Free floating, *=Exotic or non native species (Un-marked species are native or indigenous to India)
Table 3. Quantitative status of important macrophytes of Ansupa Lake, Odisha, India

| Macrophyte species                        | Total count | Total plots where recorded | Frequency | Abundance | Abundance/frequency (A/F) |
|-------------------------------------------|-------------|----------------------------|-----------|-----------|--------------------------|
| *Eichhornia crassipes* (Mart.) Solm-Laub. | 31          | 4                          | 16        | 7.75      | 0.484                    |
| *Ipomoea aquatica* Forssk.                | 17          | 3                          | 12        | 5.67      | 0.472                    |
| *Cyperus strigosus* L.                    | 14          | 2                          | 8         | 7.0       | 0.875                    |
| *Cyperus iria* L.                         | 60          | 1                          | 4         | 60.0      | 15.00                    |
| *Cyperus rotundus* L.                     | 20          | 1                          | 4         | 20.0      | 5.00                     |
| *Ludwigia adscendens* (L.) H. Har.        | 13          | 2                          | 8         | 6.5       | 0.813                    |
| *Ludwigia perennis* L.                    | 20          | 3                          | 12        | 6.67      | 0.556                    |
| *Alternanthera philoxeroides* (Mart.) Griseb. | 25        | 1                          | 4         | 25.0      | 6.250                    |
| *Salvinia molesta* D.S. Mitch             | 37          | 3                          | 12        | 12.33     | 1.028                    |
| *Salvinia minima* Baker                   | 6           | 1                          | 4         | 6.0       | 1.500                    |
| *Cyperus compressus* L.                   | 62          | 2                          | 8         | 31.0      | 3.875                    |
| *Kyllinga tenuifolia* Steud.              | 2           | 1                          | 4         | 2.0       | 0.500                    |
| *Hydrilla verticillata* (L.) Royle        | 1240        | 12                         | 48        | 103.33    | 2.153                    |
| *Ceratophyllum demersum* L.               | 4060        | 21                         | 84        | 193.33    | 2.302                    |
| *Najas faveolata* A. Br. ex Magam.        | 335         | 9                          | 36        | 37.22     | 1.034                    |
| *Nymphaeae pubescens* Willd.              | 6           | 4                          | 16        | 1.5       | 0.094                    |
| *Trapa natans* L. var. *bispinosa* (Roxb.) Makino | 8        | 1                          | 4         | 8.0       | 2.00                     |
| *Nelumbo nucifera* Gaettin.               | 57          | 16                         | 64        | 3.56      | 0.056                    |
| *Pistia stratiotes* L.                    | 11          | 3                          | 12        | 3.67      | 0.306                    |
| *Spirodela polyrhiza* (L.) Schleid.       | 54          | 4                          | 16        | 13.5      | 0.844                    |
| *Utricularia sp.*                        | 171         | 4                          | 16        | 42.75     | 2.672                    |
| *Lemma gibba* L.                          | 78          | 7                          | 28        | 11.14     | 0.398                    |
| *Azolla pinnata* R Br.                    | 29          | 5                          | 20        | 5.8       | 0.290                    |
| *Polygonum barbatum* L.                   | 38          | 1                          | 4         | 38.0      | 9.500                    |
| *Marsilea quadrifolia* L.                 | 20          | 3                          | 12        | 6.67      | 0.556                    |
| *Aponogeton natans* L. Engl. & Krause     | 5           | 1                          | 4         | 5.0       | 1.250                    |
| *Hygropyra aristata* (Retz.) Nees ex Wight & Arn | 7        | 2                          | 8         | 3.5       | 0.438                    |
| *Lindernia parviflora* (Roxb.) Haines     | 10          | 2                          | 8         | 5.0       | 0.625                    |

Plate 1. Some taxonomically important taxa from Ansupa Lake, Odisha, India. Note: A. *Oryza rufipogon*, B. *Hygropyra aristata*, C. *Ottelia alismoides*, D. *Gloriosa superba*
Plate 2. Invasive weed species of Ansupa Lake, Odisha, India. Note: A-B. *Eichhornia crassipes*, C-D. *Nelumbo nucifera*, E. *Salvinia molesta*, F. *Ceratophyllum demersum*, G. *Najas indica*, H. *Hymenachne amplexicaulis*
Besides being having these troublesome weeds, the lake also hosts many macrophytes that are used as food, fodder or medicine by the local households. Control of invasion and their management is a tedious and need multiple strategies. Management of this invasive grass must include a combination of strategies such as winter burning, herbicide application and hydroperiod control. The floating rotted macrophyte *Euryale ferox* Salisb., once occurred in the lake (recorded in October 2014) is now extinct from the lake. Implementation of physical (mechanical) methods and dredging to required depth will reduce current infested weeds and further regular monitoring, participation of both Governments agency and local community thought to restore a long term functioning of the lake.

**General comments**

Aquatic macrophytes are indispensable constituent of any wetland. They provide habitat to various aquatic fauna, act as primary producers, oxygenate water, maintain water quality, do nutrient cycling, stabilize shoreline of lakes, provide substrate for growth of algae, provide shelter to benthic fauna and breeding ground for fishes, check inflow of silt, reduce nutrient load by self utilizing and minimize development of algal blooms (Naskar 1990; Bornette and Puijalon 2009; Ansari et al. 2017). But, sometimes environments enforce and help for invasion of exotic weeds in aquatic ecosystems which negatively affect the entire ecosystem. These plants compete with native species and many times facilitate for loss or extinction of less aggressive and indigenous species (Stallings et al. 2015). In many instances they affect negatively to human activities (e.g. fishing, swimming, navigation and irrigation) and degrade the physical, chemical or biological aspects (Basak et al. 2015). In India, about 140 aquatic plants have been reported as attained the status of aquatic weeds (Naskar 1990, Gupta 2012) and many of them found in Ansupa Lake. The wetlands in India are also gradually shrinking and under severe anthropogenic pressure (Pattanaik et al. 2008; Udayakumar and Ajithadoss 2010). Regular physical visits, application of geospatial remote sensing techniques, monitoring of change in floristic composition, maintaining required depth, reducing fertilizer use in agriculture in nearby cultivation lands, creation of green coverage in surrounding barren lands can save native biota from alien species to invade many aquatic ecosystems.

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**REFERENCES**

Ansari AA, Saggoo S, Al-Ghamim SM, Abbas ZK, Gill SS, Khan FA, Dar MI, Naikoo MI, Khan AA. 2017. Aquatic plant biodiversity: A biological Indicator for the Monitoring and Assessment of Water Quality. In: Ansari AA, Gill SS, Abbas ZK, Naeem M (eds). Plant Biodiversity: Monitoring, Assessment and Conservation. CAB International, Wallingford.

Basak SK, Ali MM, Islam MS, Shahe PR. 2015. Aquatic weeds of Haor area in Kishoregonj district, Bangladesh: Availability, Threats and Management Approaches. Int J Fish Aquat Stud 2 (6): 151-156.

Bornette G, Puijalon S. 2009. Macrophytes: Ecology of Aquatic Plants. In Encyclopaedia of Life Sciences (ELS). John Wiley & Sons, Ltd., Chichester, UK.

Brundu G. 2015. Plant invaders in European and Mediterranean inland waters: profiles, distribution, and threats. Hydrobiologia 746: 61-79.

Byers EJ, Cuddington K, Jones CG, Talley TS, Hastings A, Lambrinos JG, Crooks JA, Wilson WG. 2006. Using ecosystem engineers to restore ecological systems. Trends Ecol Evol 21: 493-500.

Calvert G, Liessmann L. 2014. Wetland Plants of the Townsville-Burdekin Flood Plain. Lower Burdekin Landcare Association Inc., Ayr.

Campbell S, Higman P, Slaughter B, Schools E. 2010. A field Guide to Invasive Plants of Aquatic and Wetland Habitats for Michigan. Michigan State University Extension, East Lansing, MI, USA.

Chambers PA, Lacoul P, Murphy KJ, Thomaz SM. 2008. Global diversity of aquatic macrophytes in freshwater. Hydrobiologia 595: 9-26.

Chamier J, Schachtischneider K, Maitre DC, Ashton PJ, Wilgen BW. 2012. Impacts of invasive alien plants on water quality, with particular emphasis on South Africa. Water SA 38 (2): 345-356.

Crow GE, Hellquist CB. 2000. Aquatic and Wetland Plants of Northeastern North America. The University of Wisconsin Press, Madison, WI.

Dalu T, Clegg B, Nhwatiwa T. 2012. Aquatic macrophytes in a tropical African reservoir: diversity, communities and the impact of reservoir-level fluctuations. Trans R Soc S A 67 (3): 117-125.

Das CR, Mohanty S. 2008. Integrate Sustainable Environmental Conservation of Ansupa Lake: A famous water resource of Orissa, India. Special Issue on Dev. in Water Resources & Power Sectors in Orissa. Water Energ Intl 65 (4): 62-66.

Das NR. 2012. Introduction to Aquatic and Semi-aquatic Plants of India. Kalyani Publishers, Punjab, India.

Dodd WS. 2002. Freshwater Ecology: Concepts and Environmental Applications. Academic Press, New York.

Gerber A, Cilliers CJ, Ginkel C, van, Glen R. 2004. Easy identification of Aquatic plants: A guide for the identification of water plants in and around South African impoundments. South Africa Department of Water Affairs, Pretoria.

Ghosh SK. 2005. Illustrated aquatic and wetland plants in harmony with mankind. Standard Literature, 76, Acharya Jagadish Chandra Bose Road, Kolkata, India.

Gopal B. 1995. Biodiversity in Freshwater Ecosystems Including wetlands, Biodiversity and Conservation in India, A Status Report. Zoological Survey of India, Calcutta.

Gupta OP. 2012. Weedy aquatic plants: Their utility, menace and management. Agrobios, India.

Haines HH. 1921-1925. The Botany of Bihar and Orisha, 6 parts. London, Botanical Survey of India, Calcutta.

Margalet DR. 1958. Information theory in ecology. Year Book of the Society for General Systems Research 3: 36-71.

Mohanty S, Das CR. 2008. Community Mobilization and Participation in Implementing Integrated Sustainable Conservation of Ansupa Lake, a famous Wetland of Orissa. In: Sengupta M, Dalwani R (eds). Proceedings of Taal 2007: The 12th World Lake Conference 1240-1246.

Naskar KR. 1990. Aquatic & Semi-aquatic plants of the lower Ganga delta. Daya Publishing House, Delhi.

Oyediji AA, Abowei JFN. 2012. The Classification, Distribution, Control and Economic Importance of Aquatic Plants. Int J Fish Aquat Sci 1 (2): 118-128.

Panda SP, Sahoo HK, Subudhi HN, Sahu AK, Mishra P. 2016. Ecorffloristic diversity of Ansupa Lake, Odisha (India) with special reference to aquatic macrophytes. J Biodiv Phot 110: 537-552.

Patra S, Patra AK. 2007. Environment impact assessment of a fresh water ecosystem operating in Ansupa Lake: An urgent need for
development of fishery resources. In: Proc. Nat. Sem. on Environment pollution and its protection issues in Orissa. Organised by Department of Zoology G.S.College. Athagarh.1-2 Sept. 95-98.
Pattanaik C, Prasad SN, Reddy CS. 2008. Warning bells in Ansupa Lake, Orissa. Curr Sci 64 (5): 560.
Pielou EC. 1975. Ecological Diversity. John Wiley and Sons, New York.
Sarkar SD, Ekla A, Sahoo AK, Rashith CM, Lianthuamluaia, Roychowdhury A. 2015. Role of floodplain wetlands in supporting livelihood: A case study of Ansupa Lake in Odisha. J Environ Sci Comput Sci Eng Technol 4 (3): 819-826.
Shah MA, Reshi ZA. 2012. Invasion by alien macrophytes in freshwater ecosystems of India. In: Bhatt et al. (eds). Invasive Alien Plants: An Ecological Appraisal for the Indian Subcontinent. CAB International, Wallingford, UK.
Shannon CE, Wiener W. 1963. The Mathematical Theory of Communication. University of Illinois Press, Urbana, USA.
Simpson EH. 1949. Measurement of diversity. Nature 163: 688.
Stallings KD, Seth-Carley D, Richardson RJ. 2015. Management of Aquatic Vegetation in the Southeastern United states. J Integrat Pest Manag 6 (1): 1-5.

Udayakumar M, Ajithadoss K. 2010. Angiosperms, Hydrophytes of five ephemeral lakes of Thruvallur District, Tamil Nadu, India. Chicklist 6 (2): 270-274.
Upadhyay VP, Malviya HS, Behura S, Rout DK. 2009. Ecological methods for biodiversity assessment in EIA. Indian J Environ Ecosystem 16 (1): 157-168.
Varshney JG, Sushilkumar, Mishra JS. 2008. Current Status of Aquatic Weeds and Their Management in India. In: Sengupta M, Dalwani R (eds). Proceedings of Taal 2007: The 12th World Lake Conference. Jaipur, India. 28 October- 2 November 2007.
Wang H, Wang Q, Bower PA, Xiong W. 2016. Invasive aquatic plants in China. Aquat Invas 11 (1): 1-9.
Whitford PB. 1949. Distribution of woodland plants in relation to succession and clonal growth. Ecology 30: 199-208.
Zedler JB, Kercher S. 2004. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. Crit Rev Plant Sci 23: 431-452.
Assessing the impacts of climate variability and climate change on biodiversity in Lake Nakuru, Kenya

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Abstract. Wambui MB, Opere A, Githaiga MJ, Karanja FK, 2017. Assessing the impacts of climate variability and climate change on biodiversity in Lake Nakuru, Kenya. Bonorowo Wetlands 1: 13-24. This study evaluates the impacts of the raised water levels and the flooding of Lake Nakuru and its surrounding areas on biodiversity, specifically, the phytoplankton and lesser flamingo communities, due to climate change and climate variability. The study was to review and analyze noticed climatic records from 2000 to 2014. Several methods were used to ascertain the past and current trends of climatic parameters (temperature, rainfall and evaporation), and also the physicochemical characteristics of Lake Nakuru (conductivity, phytoplankton, lesser flamous and the lake depth). These included time series analysis, and trend analysis, so the Pearson’s correlation analysis was used to show a relationship between the alterations in lake conductivity to alterations in population estimates of the lesser flamous and the phytoplankton. Data set extracted from the Coupled Model Intercomparison Project Phase 5 (CMIPS) (IPCC Fifth Assessment Report (AR5) Atlas subset) models were subjected to time series analysis method where the future climate scenarios of near surface temperature, rainfall and evaporation were plotted for the period 2017 to 2100 (projection) for RCP2.6 and RCP8.5 relative to the baseline period 1971 to 2000 in Lake Nakuru were analysed. The results were used to evaluate the impact of climate change on the lesser flamous and phytoplankton abundance. It was noticed that there was a raise in the mean annual rainfall during the study period (2009 to 2014) which brought the increase in the lake’s surface area from a low area of 31.8 km² in January 2010 to a high of 54.7 km² in Sept 2013, indicating an increase of 22.9 km² (71.92% surface area increment). Mean conductivity of the lake also lessened leading to the loss of phytoplankton on which flamingos feed making them to migrate. A strong positive correlation between conductivity and the lesser flamous population was noticed signifying that low conductivity affects the growth of phytoplankton and since the lesser flamous depend on the phytoplankton for their feed, this subsequently revealed that the phytoplankton density could be a notable predictor of the lesser flamous occurrence in Lake Nakuru. There was also a strong positive correlation noticed between phytoplankton and the lesser flamous population which confirms that feed availability is a key determining factor of the lesser flamous distribution in the lake. It is projected that there would be an increment in temperatures, rainfall and evaporation for the period 2017 to 2100 under RCP2.6 and RCP8.5 relative to the baseline period 1971 to 2000 obtained from the Coupled Model Intercomparison Project phase 5 (CMIPS) multi-model ensemble. As a result, it is expected that the lake will further increment in surface area and depth by the year 2100 due to increased rainfall thereby affecting the populations of the lesser flamous and phytoplankton, as the physicochemical factors of the lake will alter as well during the projected period.

Keywords: Biodiversity, climate change, Lake Nakuru, Kenya

INTRODUCTION

Africa has been known as one of the most easily damaged regions in the world regarding climate change, according to the Fourth Assessment report from the Intergovernmental Panel on Climate Change (IPCC 2007). A report stated that there are some areas in Africa which evidently are highly vulnerable to climate variability and change. Increased changes and variability of different climatic factors have been forecasted by Kenya’s current climate predictions. Severe challenges to sustainable development are being propounded by climate change in Kenya, as it’s possibly a major environmental challenge of our time (Mutai et al. 2010). Focusing on effects of climate change on water resources, coastal zones, ecosystems, health, industrial activity, food and human settlements, propounds chances for improved livelihoods, business and innovation.

Various patterns of rainfall and rising temperatures have also worsened the problem of wetlands drying out, thereby threatening water availability leading to lessened agricultural production and thus accruing food insecurity due to lessening yields in crop. Various patterns of rainfall have posed threats to the renowned wildlife safaris in Kenya, and especially to one of the Seven Wonders of the World: The Mara River migration of wildebeests, which is common with tourists around the world (Climate Action Network 2009). Intermittent patterns of rain affect the wildebeests as their migration is influenced by the smell of rain, since the pattern of migration is usually timed to show a relationship between the growth of grass and annual rainfall patterns in the North. Drawing closer to March, which is characterized by a season of short dryness, the wildebeests begin migrating from Serengeti as the grass starts drying out towards the western Serengeti woodlands. By end of June when the long rains commence to decline in
Kenya, the arrival of wildebeest from the Western Serengeti is noticed in the Maasai Mara Game Reserve. Scarce feeding vegetation and the drying-up of rivers, owing to unpredictable climate, has caused huge losses in wildlife numbers (Climate Action Network 2009).

There is an extensive variety of wildlife and ecosystems in Kenya, populating in air, water and land. Biodiversity assets known in Kenya include 7,000 plant species, 315 mammals, 1,133 birds, 25,000 invertebrates (21,575 of which are insects), 191 reptiles, 692 marine and brackish fish, 180 freshwater fish, 88 amphibians and about 2,000 species of fungi and bacteria (NEMA 2009a). Kenya boasts a large population of mammalian species’ ranking it third in Africa, with fourteen of these species endemic to the country (IGAD 2007). Large mammals such as the African elephant (Loxodonta africana), leopard (Panthera pardus), black rhino (Diceros bicornis), African lion (Panthera leo) and buffalo (Syncerus cafer) have made the country become popular due to their diverse nature (NEMA 2009a). According to the IUCN Threat Criteria (2008), 146 plant species of the 7000 found in Kenya have been assessed with 103 being classified as threatened (vulnerable, endangered or seriously endangered) (NEMA 2011).

In Kenya, threats to biodiversity have been on the increment over the past decades due to human-wildlife conflicts, habitat loss, population increment and infrastructure development, global climate change, pollution, biopiracy, poaching and overexploitation, invasive alien species and biosafety concerns (Government of Kenya (GoK), National Environment Management Agency (NEMA 2011)). In this regard, safeguarding these biodiversity will be critical to securing livelihoods resulting to reduced levels of poverty - reflecting a population of 46.6 percent - suggesting a nine percent alteration if the social equity scales are to be attained as projected by the Vision 2030’s social pillar (NEMA 2011).

Provided crucial coping, mitigation and adaptation approaches are realized, future climate variability and climate change impacts can be avoided, delayed or reduced. About US $500 million per year was needed in Kenya to address the climate change effects by 2012 (Stockholm Environment Institute 2009). US $1-2 billion per year was the amount this figure was forecasted to raise to by 2030 (Stockholm Environment Institute 2009). The collective effect of impacts of climate change will limit the realization of Vision 2030 targets, unless there is an urgent institutionalization of effective adaptation and mitigation mechanisms. As such, in order to tackle climate change, a range of policy instruments need to be formulated. A national policy on climate change need to be formulated and a climate change law further enacted, recognizing that the National Climate Change Response Strategy (NCCRS) was finalized in 2010. The country will not only be economically affected by the impacts of climate change but also its biodiversity heritage.

The main objective of the research was to evaluate the impacts of climate variability and climate change on Lake Nakuru’s biodiversity, Kenya, i.e., (i) to estimate the trends of past and present climatic records, and especially the temperature, rainfall and evaporation, of Lake Nakuru basin in order to understand the causes of increased lake levels. (ii) to show a relationship between alterations in lake conductivity to alterations in population estimates of aquatic species especially the phytoplankton and the lesser flamingos of Lake Nakuru basin. (iii) Evaluate in light of future climate projections, especially temperature, rainfall, and evaporation, the likely impacts of climate change on Kenya’s biodiversity especially the lesser flamingos and phytoplankton in Lake Nakuru basin.

MATERIALS AND METHODS

Area of study

The study site was Lake Nakuru. It was chosen because it is one of the most important habitats for the flamingo species and also one of the important tourist destinations in Kenya. Lake Nakuru National Park, Kenya is located between 0°19' S and 36°07 E, approximately 3km South of Nakuru town, Kenya. It lies in a graben between Lion Hill fracture zone in the east and a series of east downthrown step-fault scarp leading to the Mau Escarpment to the west.

Lake Nakuru extends in the N-S direction in the trend of the axial rift faults as shown by Figure 1. It includes other chains of alkaline-saline lakes in the eastern arm of the Rift Valley, Kenya. Existing more than twelve million years, one of the earth’s spectacular geological formations was formed by the catchment and its landforms which included rifts, cliffs, mountains, volcanoes and lakes (Odada et al. 2006). Progressions of characteristics and features that describe Lake Nakuru have been influenced by climate, evolutionary history and Geography. Levels of productivity and successful establishment of species have been ascertained by these features which set in motion the chemistry of the lakes’ water. The ecosystem of the lake is made unique by the chemistry of the alkaline water which depends on the larger catchment for sustenance and independent of its immediate environment for it functions. White salt filets swirling with dust devils are sometimes created when there are enormous water body reductions resulting from alterations in the surface area of the lake.

Data type

Data used in this study included climatic data comprising of mean annual temperature, mean annual rainfall mean annual evaporation and the Coupled Model Intercomparison Project Phase 5 (CMIP5) Representative Concentration Pathways (RCP2.6 and RCP8.5) near surface temperature, rainfall and evaporation data. Lake data comprised of conductivity, lake levels, surface area and depth. Flamingo data comprised of the lesser flamingo population. Below is a detailed description of the data types and their sources.

Procedures

In this section, the methods that were used in the study for data collection, organization and analysis based on the specific objectives of the study are propounded.
Study design and sample size determination

Sample and sample size identification was purposive and quantitative. Four sites Nderit, Makalia, Baboon Cliff and Lion Hill, were selected as sampling sites/stations for the collection of water samples which were used to ascertain the levels of phytoplankton population densities, species composition and conductivity measurements in the lake in 2014. The sampling sites were largely ascertained by their accessibility as the lake was quite flooded at the time leading to the loss/damage of the road infrastructure which limited access to other sites. Water samples from the lake were collected in four replicates, fortnightly, in August 2014 and the first half of September 2014. The study sought to investigate the impact of climate variability and climate change on Lake Nakuru’s biodiversity on the following indicators: (i) Lake levels (surface area and depth), (ii) Conductivity levels, (iii) Alteration in species populations of the phytoplankton and the lesser flamingos.

Data sources

Field visits and preliminary assessments. The water samples collected were used to ascertain the levels of phytoplankton population densities, species composition and conductivity measurements in the lake in 2014. These measurements were compared with data acquired from the Kenya Wildlife Service database for the period 2009 to 2014. Samples were collected in sterile bottles and transported in a cool box to the laboratory at the School of Biological Sciences, Chiromo Campus, University of Nairobi, where ex situ measurements of conductivity and phytoplankton concentration were conducted. Before analysis, the samples were stored in a fridge in the laboratory.

A Hanna Multi-parameter Water Analyzer Model HI 9828 was used to measure the conductivity of the water samples collected. The mean value of the four replicates was ascertained for each sampling site which was used to compute the mean conductivity of the lake. To ascertain the phytoplankton cells concentration and species identification one replicate from each site was randomly selected. 1 µL was taken from the bottle and suspended and centrifuged in 100 µL sterilized water. 1 µL of this suspension was placed on a glass slide and noticed under a LEICA DM500 microscope where the number of individuals in the field of view (quadrant) were counted and identified. This process was replicated for the other samples.

Populations of lesser flamingos. Data on population estimates of the lesser flamingos in the lake for the period 2009 to 2014 was collected from the KWS Bi-annual Waterfowl Count Report- Kenya Rift Valley Lakes ascertained using a modification of a method as described by (Pomeroy & Dranzoa, 1997). The information was used to ascertain the recent population trends and movement patterns of lesser flamingo in the context of flooding and the extensive dilution of the lake and show a relationship
between the alterations in lake conductivity to alterations in the population estimates of the phytoplankton and lesser flamingo for the period 2009 to 2014. The data was based on records of the January water bird counts that are conducted jointly by the National Museums of Kenya and Kenya Wildlife Service.

**Alternations in the lake levels.** Data to ascertain the alternations in the lake surface area and depth was obtained from (Onywere et al. 2013) and the Kenya Wildlife Service records respectively. Documentation of the alternations in the lake surface area was made using Geographic Information System (GIS) digital techniques and information extraction and representation from Landsat satellite image data for January 2010, May 2013 and September 2013 and October 2013 (Onywere et al. 2013), whereas monthly measurements of the depth of the lake was collected from KWS. This had been ascertained from the readings of a staff gauge located at the lake centre.

**Physicochemical characteristics of water (phytoplankton concentration and conductivity).** The physicochemical qualities of water (phytoplankton concentration and conductivity) for the period 2009-2013 were obtained from the Kenya Wildlife Service (KWS) database. Monthly measurements of conductivity and concentration of phytoplankton in lake water had been ascertained based on monthly analysis of water taken from the lake centre. Conductivity had been ascertained using a pH meter. The concentration of phytoplankton had been ascertained using the Sedgwick-Rafter counting chamber as described by Kimberly (1999).

**Noticed climate data.** The climatic data (Rainfall, temperature, evaporation) for the period 2009 to 2014 was collected from the Kenya Meteorological Department, based on monthly data from the Nakuru Meteorological Station - 9036261(0.28°S, 36.1°E), located 3km north of the lake at the Nakuru Agricultural show grounds.

**Climate projection data sets.** In this study, the projected alternations in near surface temperature, rainfall and evaporation for Lake Nakuru were extracted from the Coupled Model Intercomparison Project Phase 5 (CMIP5) multi-model ensemble (IPCC Fifth Assessment Report (AR5) Atlas subset) models. The output data were extracted as a relative alteration from 1971 to 2000 (baseline) to, 2017 to 2100 (projection) under two scenarios, namely, the RCP2.6 and RCP8.5 scenarios (Taylor et al. 2012). The RCP2.6 and RCP8.5 represent ‘low’ (RCP2.6) and ‘high’ (RCP8.5) scenarios featured by the radiative forcings of 2.6 and 8.5 Wm−2 by 2100, respectively. The CO2 equivalent concentrations in the year 2100 for RCP 2.6 and RCP 8.5 are 490 ppm and 1370 ppm, respectively (Moss et al. 2010). RCP2.6 and RCP8.5 were chosen for this study as RCP2.6 describes an all-out effort to limit global warming to below 2°C with emissions lessening sharply after 2020 and zero from 2080 onward, whereas RCP8.5 describes a business-as-usual scenario with accruing greenhouse gas emissions over time, leading to high greenhouse gas concentration levels.

These Representative Concentration Pathways (RCPs) are among four new GHG concentration developed scenarios set containing emission, concentration and land-use trajectories which have been adopted by the IPCC Fifth Assessment Report (AR5) (Moss et al. 2010; Van Vuuren et al. 2011; IPCC 2014). They describe possible climate futures explaining the possible range of forcing values up to the year 2100, with respect to the situation before industrialization. RCP2.6 and RCP8.5 were chosen for this study as they

**Data quality control**

Data quality control was conducted to ensure that the data sets were devoid of missing values, consistent, uniformly entered and arranged to facilitate further processing. The data was then subjected to various statistical computations.

**Homogeneity test.** Most long-term climatological data records have been affected by a number of non-climatic factors that make these records unsuitable for comparison over long time periods and between different stations. These relate to alterations that can affect instruments, site, or procedures and methods in the observations and data processing. These factors are caused by alterations in: instrumentation, observation practices, location of station, and formulae used for means calculation, and changing the environment of the station. While some alterations make critical discontinuities, others, particularly alterations around station environment, due for example to, urbanisation, causes data biases which are gradual leading to time series biases and studied climate misinterpretations. In this study, the cumulative mass curve technique described in the subsection below was used to test for data homogeneity.

**Mass curve.** Mass curve analysis entails plotting of cumulative climatological data records against time to depict the homogeneity. The patterns of these graphs can be used to test for the quality of the records. A single straight line indicates a homogeneous record whereas heterogeneity tendency is indicated by existence of more than one line fitted to the graphical plots of the cumulative data. For the heterogeneous records, correcting the heterogeneity would be the next step. Double mass curves are commonly used to adjust heterogeneous records whose principles are similar to those of mass curves. In this study, the single mass curve technique was used to test the data consistence where cumulative rainfall and temperature data was plotted against time to depict the homogeneity. A straight line graph depicted homogeneous data.

**Time series analysis**

Time series is the organization of statistical data in chronological order; in order with its time of occurrence. In this study a plotting of the annual means for rainfall, temperature and evaporation data for the period 2000 to 2014 using graphical method was undertaken. In addition, annual data means for lake depth, lesser flamingo population, conductivity, and phytoplankton levels for the period 2009 to 2014 were also plotted.

In order to ascertain the projected alterations in near surface temperature, rainfall and evaporation for Lake Nakuru, data extracted from the Coupled Model Intercomparison Project Phase 5 (CMIP5) multi-model
ensemble (IPCC Fifth Assessment Report (AR5) Atlas subset) models were plotted using the KNMI (2015) to analyse the data for the period 2017 to 2100 for RCP2.6 and RCP8.5 relative to the baseline period 1971-2000.

The trend is characterized by the long term movement that is either represented by a growth or decline in a time series through a lengthy period of time. The trend in time series in this study, graphical method was used to ascertain the past and current trends of climatic parameters (temperature, rainfall and evaporation), and also for the physicochemical characteristics of Lake Nakuru (conductivity, phytoplankton, lesser flamingos and the lake depth).

Standard error of the mean was used to provide information about the distribution of the values within the trends as shown by Equation (1).

\[ \sigma_M = \frac{\sigma}{\sqrt{N}} \]  
\[ \text{Equation (1)} \]

Where, \( \sigma_M \) is the standard error of the mean, \( \sigma \) is the standard deviation of the original distribution and \( N \) is the sample size (the number of counts each mean is based upon). Specifically in this study, the error bars were fitted graphically to evaluate whether there was a notable difference between the data sets. While a larger sample size suggests a smaller standard error of the mean, overlapping error bars implies that the difference is usually not notable. However, when the error bars do not overlap, it suggests that the difference is notable.

**Correlation analysis**

The Pearson Correlation coefficient (\( r \)), given in equation (2), was used to quantify the degree of relations between pairs of study variables. It is used extensively as a measure of the degree of linear dependence among two variables. If two variables ‘x’ and ‘y’ are so related, where, ‘x’ is the conductivity of the lake and where, ‘y’ is represented by either the phytoplankton or the lesser flamingos, the variables in the magnitude of one variable tend to be accompanied by variations in the magnitude of the other variable, they are said to be associated. Therefore, correlation as a statistical tool helps to ascertain whether or not two or more variables associate and if they are associated, the degree and direction of their correlation.

\[ r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{n(\Sigma x^2) - (\Sigma x)^2}[n(\Sigma y^2) - (\Sigma y)^2]} \]  
\[ \text{Equation (2)} \]

Where, \( r \) is the Pearson correlation coefficient, \( N \) is the sample size, \( \Sigma xy \) is the sum of the products of paired scores, \( \Sigma x \) is the sum of x scores, \( \Sigma y \) is the sum of y scores, and \( \Sigma x^2 \) is the sum of squared x scores.

The student T-test was used to test for the significance of the correlation coefficient. The computed t-statistic derived from Equation (3), was compared with the tabulated t-value of the student t-distribution at the \( n-2 \) degrees of freedom and 5% significance level.

\[ t_{n-2} = r \sqrt{\frac{n-2}{1-r^2}} \]  
\[ \text{Equation (3)} \]

Where, \( n \) represents the length of the data that were used, \( n-2 \) is the degree of the freedom, \( n-2 \) is the computed t-statistic and \( r \) is the Pearson correlation coefficient.

Correlation coefficient was deemed to be notable if the computed value of \( t \) was greater than the tabulated value at the 5% significance level. This is usually conducted to ascertain whether the linear relationship in the sample data is strong enough to use to model the relationship in the population.

**RESULTS AND DISCUSSION**

**Data quality control**

In this section, results of data quality control are propounded and their suitability for the study established. Specifically this section propounds results of the homogeneity test. The Figures 2 and 3 show simple mass curves for rainfall and temperature respectively. It can be noticed from Figures 2 and 3 that the rainfall and temperature data sets were homogeneous, owing to the resistant straight line plots. It can be noted that generally the rainfall has been gradually accruing leading to an increment in the surface runoff, most of which subsequently ended up in the lake.

![Figure 2. Single mass curve, cumulative annual rainfall](image1)

![Figure 3. Single mass curve, cumulative annual temperature](image2)
Past and present climatic record of Lake Nakuru from 2000 to 2014

Trend analysis of climatic data

Trends in rainfall patterns from 2000 to 2014. There has been marked variability of the mean annual rainfall patterns of Lake Nakuru basin with major rainfall intensification in the years 2000 to 2001 and 2009 to 2010, with the highest (120 mm) being recorded in 2010 (Figure 4).

Trends in temperature patterns from 2000 to 2014. Mean annual temperatures has been on a lessening trend during the period 2000 to 2014, with the highest temperatures being recorded in 2000 (26.6°C) and in 2009 (27°C) (Figure 5). Evaporation in the Lake Nakuru basin shows a declining trend over the study period (Figure 6). However, the noticed decrement in evaporation from the year 2009 is consistent with the increment in rainfall noticed in Figure 4 and temperature decrement noticed in Figure 5.

Alterations in the lake levels (depth and surface area) 2009 to 2014. Time series of Lake Nakuru levels (depth). Lake Nakuru levels have been rising over the years 2009 to 2014 (Figure 7). As seen in Figure 7, the mean depth of the lake rapidly increased during the study period (2009 to 2014). This could have been caused by increased rainfall during the study period which led to increased surface runoff and direct rainfall into the lake. The increased water levels led to the flooding of the lake which further lowered the conductivity of the lake as more fresh water was added into it.

Alterations in the lake surface area

Lake Nakuru’s surface area increased from an area of 31.8 km² in January 2010 to a high of 54.7 km² in Sept 2013 (Figure 8 and 9), an increment of 22.9 km² (71.9%). This led to the submergence of 60% of the transport infrastructure in Lake Nakuru Nationa Park and the park’s main gate, during this period, thereby displacing wildlife. At the highest level, the lake expanded and submerged areas that have never been recorded in the last 100 years (Figure 9). The extent of the flooded area and the impacts are illustrated in the image data and digitized maps shown in Figure 10.

Conductivity, phytoplankton levels and the lesser flamingos populations

Conductivity levels

The mean conductivity of Lake Nakuru lessened from the period 2009 to 2014 (Figure 11).
This coincided with the beginning of the rains from the year 2010 as shown in Figure 4. The declining conductivity of the lake could result into loss of phytoplankton (reduction in food supply) upon which the lesser flamingos feed. This could eventually lead to the migration of the lesser flamingos from the lake. This is due to the fact that as more fresh water was added in to the lake, it lowered the conductivity of the lake because fresh water has low conductivity and the increment in water levels dilutes mineral concentrations.

**Phytoplankton levels**

The phytoplankton levels in Lake Nakuru were quite variable for the years 2009 to 2014 as shown by Figure 12. Notably, however, there was a general reduction in the phytoplankton levels which coincided with the onset of the rains from the year 2010 as shown in Figure 4. Phytoplankton levels lessened from 606 Units/mL in 2010 to 187 Units/mL in 2012. However, there was an increment in the phytoplankton levels to 321 Units/mL in 2013 which could have been caused by alterations in phytoplankton species composition and diversity that in turn affected their abundance due to alterations in the chemical and physical properties of the water (Kihwele et al. 2014).

**Lesser flamingos population**

The number of lesser flamingos drastically lessened from the beginning of the rains in 2010 (Figure 13) from 41,592 in 2010 to 10,168 in 2011 and further lessened to 110 in 2012. This pattern follows that of lessening phytoplankton levels shown in Figure 12.
that there was a notable positive correlation between phytoplankton and the lesser flamingo (r=0.731, p=0.099).

Table 1. Correlation of alterations in lake conductivity to alterations in population estimates of the phytoplankton in Lake Nakuru during the study period (2009 to 2014)

| Conductivity in (mS/cm) | Phytoplankton (Units/mL) |
|------------------------|--------------------------|
| 1                      | .437                     |
| Pearson correlation    |                          |
| Sig. (2-tailed)        | .386                     |
| N                      | 6                        |

Table 2. Correlation of the alterations in electrical conductivity to alterations in population estimates of the lesser flamingo in Lake Nakuru from 2009 to 2014

| Conductivity in (mS/cm) | Lesser flamingo water bird |
|------------------------|-----------------------------|
| 1                      | .767                        |
| Pearson correlation    |                             |
| Sig. (2-tailed)        | .075                        |
| N                      | 6                           |

Correlation between alterations in lake conductivity and alterations in population estimates of the phytoplankton and the lesser flamingo

The findings in Table 1 showed that there was a nonsignificant positive correlation between conductivity and phytoplankton, (r=0.437, p=0.386). The findings in Table 2 showed that there was a notable positive correlation between conductivity and the lesser flamingo, (r=0.767, p=0.075). The findings in Table 3 also showed

Figure 9. Lake Nakuru highest water level in September 2013 (Source: Onywere et al. 2013)

Figure 13. Trend in the number of lesser flamingos for the period 2009 to 2014
Figure 10. Time series extent of flooding in Lake Nakuru from a low area of 31.8 km² in January 2010 to a high of 54.7 km² in Sept 2013, a total increment of 22.9 km² (71.9%) (Onywere et al. 2013)

Table 3. Correlation of the alterations in the population estimates of the lesser flamingo in Lake Nakuru to alterations in the population estimates of the phytoplankton from 2009 to 2014

| Lesser flamingo | Phytoplankton | Pearson correlation | Sig. (2-tailed) |
|-----------------|---------------|---------------------|-----------------|
| water bird      |               |                     |                 |
| Lesser flamingo |               | .731                | .099            |
| water bird      |               |                     |                 |
| N               | 6             | 6                   |                 |

Projections of the climatic data (temperatures, evaporation and rainfall) for the period 2017-2100

Future climate scenarios of Lake Nakuru comprising near surface temperature, rainfall and evaporation were plotted for the period 2017 to 2100 (projection) for RCP2.6 and RCP8.5 relative to the baseline period 1971 to 2000. The results obtained are sequentially propounded in the subsections that follow.

Near surface temperature projections

Future alterations in annual temperature for Lake Nakuru under RCP2.6 and RCP8.5 for the period 2017 to 2100 relative to baseline period 1971 to 2000 are propounded in Figure 14 and 15 respectively.

Temperature projections indicate an accruing trend with a 1.2°C increment for the 2071 to 2100 mean alterations for RCP2.6 (Figure 14) whereas there is a 4.8°C increment for the 2071 to 2100 mean alterations for RCP8.5 (Figure 15). The likely causes of the accruing trend of temperature under both RCP2.6 and RCP8.5 could be due to accruing levels of greenhouse gas concentrations in the atmosphere during the projected period. Notably however, the rate of temperature increment in RCP2.6 is lower than that of RCP8.5.

Rainfall projections

Future alterations in rainfall for Lake Nakuru under RCP2.6 and RCP8.5 were plotted for the period 2017 to 2100 relative to baseline period 1971 to 2000 are shown in Figures 16 and 17 respectively.

The rainfall projection from RCP2.6 and RCP 8.5 show a 10% and 20% increment in rainfall for the 2071 to 2100.
Evaporation projections

Future alterations in evaporation for Lake Nakuru under RCP2.6 and RCP8.5 for the period 2017 to 2100 relative to baseline period 1971 to 2000 are shown in Figures 18 and 19.

Relative evaporation is projected to raise by 10% and 20% for the 2071 to 2100 mean changes for RCP2.6 and RCP8.5 respectively.

![Figure 14](image1.png)
**Figure 14.** Near surface temperature projection for RCP2.6 for the Lake Nakuru area from 2017 to 2100 shows a 1.2°C increment for the 2071 to 2100 mean alterations

![Figure 15](image2.png)
**Figure 15.** Near surface temperature projection for RCP8.5 for the Lake Nakuru area from 2017 to 2100 shows a 4.8°C increment for the 2071 to 2100 mean alterations

![Figure 16](image3.png)
**Figure 16.** Rainfall projections for RCP2.6 for Lake Nakuru

![Figure 17](image4.png)
**Figure 17.** Rainfall projections for RCP8.5 show a 20% increment in rainfall in Lake Nakuru area for the 2071 to 2100 mean alterations

![Figure 18](image5.png)
**Figure 18.** Relative evaporation alteration for RCP2.6 for the Lake Nakuru for 2071 to 2100

![Figure 19](image6.png)
**Figure 19.** Relative evaporation alteration for RCP8.5 for Lake Nakuru

Discussions

As drawn in Figure 4, it can be concluded that rainfall has increased since 2010 and this may have greatly affected the accruing water levels in lakes as shown by the images obtained from (Onywere et al. 2013). Figure 7 also depicts that the depth of the lake has gradually increased since the beginning of the rain in 2010. This can be strongly associated with increased surface runoff and lake catchment improvements from Njoro, Makalia, Larmudiac and Enderit Rivers as well as from direct rainfall to lakes.
Figures 5 and 6 depict that temperature and evaporation have been raising from 2007 to 2009, the same period when there was little/no rain, and drastically lessened from 2009 to 2010, the beginning of the rainy season. According to Trenberth (2011), an increment in temperature usually leads to an increment in evaporation and therefore drought. Heating about 7% per 1 °C raises the water holding capacity of the atmosphere. This is noticed in studies with temperatures and evaporation raising in 2007 to 2009, which increased the capacity to water retaining of the atmosphere, and thus the beginning of rain in 2009.

According to the IPCC (2007), climate change is clearly more and easily accessed by temperature, although atmospheric moisture changes, atmospheric circulation and rainfall are also ascertained because the overall climate system is generally influenced. The capacity to withstand atmospheric moisture is made increment as temperature becomes higher at a rate of about 7% per °C (Trenberth et al., 2003). Collectively, changes in the hydrological cycle are influenced, in particular, the characteristics of rainfall (type, intensity, amount, duration, frequency) and extremes (Trenberth et al., 2003). The increased water vapor convergence guides to heavier rainfall but a reduction in duration and/or frequency in the weather system, given that the total amount changed a little. Therefore, it can be concluded that a slight increment in temperature induces a tighter hydrological cycle because the evaporation rate is also increased which has a direct impact on cloud formation, since intense rainfall is affected as atmospheric water containment capacity increases, as noticed in 2010.

Seasonal hydrological budget changes greatly affect endorheic lakes that may be extreme at a time, resulting in drastic algal biomass accidents and major changes in community composition as has been noticed in Lake Nakuru.

As more water is added to the lake, it liquidized the mineral concentration thereby lessened the electrical conductivity of the lake. Fresh water has low conductivity. According to the study made in Figure 11, the conductivity level began to decline at 2010, after the start of the rain. The relationship between lake water conductivity and lake depth examined in this study reflects the cycle of concentration and dilution of the lake due to evaporation during the dry season followed by replenishment from river in-flow and water run-off during the wet season. These hydrological cycles have a profound effect on aquatic biota in the lake (Githaiga, 1997).

The correlation coefficient in Table 1 showed changes in lake conductivity and corresponding changes in phytoplankton population estimates. The undistinguished coefficient between conductivity and phytoplankton (r = 0.437, p = 0.386) reflects the cycle of lake dilution due to replenishment from the river in-flow and water run-off during the rainy season. Some aquatic species adjusted to life in highly alkaline water at Lake Nakuru and achieved a very high level of biomass that serve as food for the main feeder. The species of blue green algal, *Arthrospira fusiformis* is one such species and it is the main food of the lesser flamingos. Thus, when the level of conductivity of the lake lessened, the rate of phytoplankton in the lake also lessened because the conditions were not conducive for them to bloom.

Table 2 shows that the conductivity had a strong positive correlation, with a lesser flamingo (r = 0.767, p = 0.075). This entails that low conductivity impacts the growth of phytoplankton by making an undesirable environment for raising phytoplankton. Because the lesser flamingo relies on phytoplankton for their feed, then it suggests that the denseness of phytoplankton can be a notable predictor of the lesser flamingo occurrence in Lake Nakuru. The noticed correlation which is high and strong (r = 0.731, p = 0.099) between phytoplankton and lesser flamingo showed in Table 3 confirms that in saline lakes, the distribution of lesser flamingo is affected by the availability of feed.

Figures 14 to 19 shows an increment in temperature, rainfall and evaporation for the period 2017 to 2100 under RCP2.6 and RCP8.5 relative to the baseline period 1971 to 2000 acquired from the Model Combined Model Intercomparison Phase 5 (CMIP5) multi-model ensemble.

As you will notice, the rising rate in temperature, rainfall and relative evaporation in RCP8.5 are looked to be higher than in RCP2.6. This is assigned to the fact that RCP8.5 is qualified by a business-as-usual scenario with rising greenhouse gas emissions over time, conducing to high levels of greenhouse gas concentrations equated to RCP2.6 which exemplifies an all-out attempt to restrict warming global to below 2°C with emissions declining sharply after 2020 and zero from 2080 onwards.

Based on the rainfall projection (Figures 16 and 17), it is estimated that the average depth of the lake will raise over time because the replenishment is strongly affected by rainfall, which is also positively associated with the temperature. Therefore, an increment in rainfall will result in an increment in discharge during the projection period. As explained before, a slight increment in temperature induces a stronger hydrological cycle, and therefore, because the projection indicates an increment in temperature level, it is estimated that the hydrological cycle will be very strong during the projection period, which assumes that ultimately, the hydrological cycle will be altered, resulting in more intense rain, characterized by thunderstorms (Trenberth 2011).

The raising replenishment during the projection period will make the level of conductivity in the lake to lessen, indicating the increased concentration and dilution cycle of the lake due to evaporation during the dry season followed by replenishment by the river in-flow and water run-off during each wet season. The decrement in conductivity levels will consequently alter the condition of the lake making an unfavorable environment for phytoplankton thrives, thereby cutting down the handiness of feed for lesser flamingos and finally, cutting down the amount of lesser flamingos in lakes due to their migration to other lakes which harbor the food supply of their choice and with the appropriate living conditions.

Based on the fact that the lake area has a negative rain fall/evaporation deficit, the raising temperature will induce a higher evaporation rate and therefore higher conductivity
due to evaporative concentration, during the projected period.

Conclusions

The study discovered that climate change and climate variability can cause substantial effects on saltwater lakes by making modifications in their physicochemical traits. This has been proved by the variations in rainfall and temperature which affects the phytoplankton availability, ascertained by the chemical and physical traits of the lake. There were fluctuations on the lesser flamingos’ population due to climate variability. These were because of the alterations in rainfall that influenced the physicochemical composition, lake depth, and the surface location of the lake which ultimate effect is discovered in the abundance of the phytoplankton (foods for the lesser flamingos). This study indicates that, the shift and succession in phytoplankton species has a relation with the variations in the physicochemical elements of the lake, especially the conductivity which are greatly affected by the variability of climate. The study also proposes that population dynamics of the lesser flamingos may be affected by using availability of their essential meals, *Arthrospira fusiformis* which is in turn influenced by physicochemical properties of water and also by weather variability. Based on future projections, it is hoped that the lake will maintain growing in surface area and depth by the year 2100 because of increased thereby influencing the populations of the lesser flamingos and phytoplankton, as the physicochemical elements of the lake will also change in the course of the projected period.

REFERENCES

CBD [Convention on Biological Diversity]. Convention on Biological Diversity, United Nation 1992. http://www.cbd.int/doc/legal/cbd-en.pdf (12 April 2014).

Climate Action Network. 2009) Kenya: A country of Growing Despair: Frontline View. Voices from communities in developing countries most affected by escalating climate change impacts. Available at: climatedata.org/eco/barcelona-2009-ecos/Voice2.pdf

Githaga JM. 1997. Utilization patterns and inter-lake movements of the Lesser Flamingo and their conservation in Saline Lakes of Kenya. In: Howard, G. (ed.). Conservation of 166 the Lesser Flamingo in Eastern Africa and beyond. IUCN, Nairobi Kenya

NEMA. 2011. Kenya State of the Environment and Outlook 2010. National Environment Management Agency, Government of Kenya.

IGAD. 2007. IGAD Environment Outlook. Intergovernmental Authority on Development (IGAD), Djibouti.

IPCC. 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.

IPCC. 2012. Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.

Kihwele ES, Lugomela C, Howell KM. 2014. Temporal changes in the Lesser Flamingos population (*Phoenicopterus minor*) in relation to phytoplankton abundance in Lake Manyara, Tanzania. Open J Ecol 4: 145-161.

Kimberly MJ. 1999. Microcystins in components of twelve New Hampshire lakes of varied trophic status. UNH Centre for Freshwater Biology Research, 1 (4): 45-56.

KNMI. 2015. KNMI Climate Change Atlas. 2015. accessible from: https://climexp.knmi.nl/plot_atlas_form.py

KWS. 2009; 2010; 2011; 2012; 2013; 2014. KWS Bi-annual Waterfowl Count Report-Kenya Rift Valley Lakes

Moss RH, Edmonds JA, Hibbard KA, Manning MR, Rose SK, Van Vuuren DP, Wilbanks TJ. 2010. The next generation of scenarios for climate change research and assessment. Nature 463 (7282): 747-756.

Mutua CC, Ochola S, Mukiria H, Gachimbi LN, Otiono M, King’uyu SM, Marigi SN. 2010. Climate Change and Variability. State of the Environment in Kenya Report. Ministry of Environment and Natural Resources, Nairobi, Kenya.

NEMA. 2009. The 4th National Report to the Conference of Parties of the Convention on Biological Diversity, Nairobi, Kenya.

Odada EO, Rami J, Nderi R. 2006. Lake Nakuru: experience and lessons learned brief. Lake Basin Management Initiative: Main Report, 299-321.

Pomeroy DE, Dranoviz C. 1997. Methods of studying distribution, diversity and abundance of birds in East Africa—some quantitative approaches. African J Ecol 35: 110-123.

Stockholm Environment Institute. 2009. The Economics of Climate Change in Kenya, SEL Oxford

Taylor KE, Stouffer RJ, Meehl GA. 2012. An overview of CMIP5 and the experiment design. Bull Amer Meteorol Soc 93: 485-498.

Trenberth K.E, Dai. A, Rasmussen R.M, Parsons D.B. 2003. The changing character of precipitation. Bull Amer Meteorol Soc 84: 1205-1217.

Trenberth K. 2011. Changes in precipitation with climate change. Clim Res 47: 123-138.

Van Vuuren DP, Riahi K, Moss R, Edmonds J, Thomas A, Hibbard K, Hurtt GC, Kram T, Krey V, Lamarque JF, Masui T. 2011. The representative concentration pathways: an overview. Climate Change 109: 5-31.
Diversity and distribution of immature vectors of malaria and rift valley fever in habitats along an altitudinal gradient in Barigo, Kenya

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Abstract. Dancan K. 2018. Diversity and distribution of immature vectors of malaria and rift valley fever in habitats along an altitudinal gradient in Baringo County, Kenya. Bonorowo Wetlands 2: 25-32. Malaria and RVF are two diseases whose onset of epidemics leads to massive losses in human lives. Infected Anopheles mosquitoes transmit Plasmodium parasites that cause malaria while infected floodwater Aedes species are responsible for primary transmission of RVF viruses. The high mobility of adult mosquito species has made interventions targeting their behavior are rendered ineffective. Thus, interventions that target immature stages more advantageous. For effective implementation of immature stage based control strategies, information on their diversity and distribution in various habitats distributed along altitudinal gradients is important. This study investigated the diversity and distribution of malaria and RVF mosquito vectors at immature stages along an altitudinal gradient in Baringo County, Kenya during the short rains season. The species identified in the entire study area (800 m to 2300 m above sea level) were Culex quinquefasciatus, Cx. annulioris, Cx. pipiens, Cx. poicilipes, Cx. tigripes, Anopheles pharoensis, An. gambiae s.l, An. coustani, An. funestus, and Aedes taylori. Altitude was divided into three classes; 800 m to 1300 m, 1301 m to 1800 m and 1801 m to 2300 m. Aedes taylori and Cx. tigripes were only in the 1801 m to 2300 m altitudinal class while An. funestus was only in the 800 m to 1300 m altitudinal class. The altitudinal class between 1801 m to 2300 m had the lowest Shannon-wiener diversity index (H’ = 0.9836) of species (9species). Comparison of mosquitoes collected in habitats in different altitudinal classes revealed variations in the respective species counts (χ² = 127.47; p-value < 0.001). The only species whose distribution showed correlation with altitude was An. pharoensis (r = -0.40; t32 = -2.50; p = 0.02). The highest species diversity was recorded in river banks where the water was clear and vegetation present. Stepwise regression analysis revealed that suitability of a habitat for vector breeding was mainly dictated by water quality and the presence of vegetation. The results in this study reveal the need for continuous monitoring of vectors not only in the low land areas but also in the highland areas to avoid sudden epidemics of malaria and RVF.

Keywords: Aedes, altitudinal gradient, Anopheles, Culex, diversity, immature vectors

INTRODUCTION

Malaria and Rift Valley Fever are some of the vector-transmitted diseases that have claimed many lives in tropical Africa (Woods et al. 2002; WHO 2013b). Malaria is caused by protozoan parasites of the genus Plasmodium transmitted by infected female mosquitoes of the genus Anopheles. It is currently the leading cause of mortality and morbidity in many countries with 90% of the mortalities in Africa (WHO 2013a). In Kenya, 20% of reported child mortalities under 5 years are as a result of malaria (KEMRI 2014). Baringo County in Kenya is one of the malaria endemic zones and experiences seasonal epidemics.

Rift valley fever, the second vector transmitted a Phlebovirus of the family Bunyaviridae causes disease. Trans-ovarian transmission maintains rift valley fever in floodwater Aedes mosquitoes. Outbreaks are associated with heavy, prolonged rainfall which is often associated with the El Niño phenomena. Secondary transmission in epidemics is mainly by female Culex mosquitoes and biting flies (Swaneoel et al. 2011; El Vilaly et al. 2013). In 2006 to 2007 Kenyan epidemic, a total of 684 cases were reported including 155 human deaths (23%). Amongst the 684 cases, about 183 were in the rift valley (WHO 2007), which were part of Baringo district (now Baringo County).

Like other insect species, the distribution range of many insect disease-vectors including the Anopheles, Culex and Aedes species, is defined by climatic factors that favor their respective physiological functions (Githeko et al. 2000). Factors such as temperature, humidity, and precipitation tend to vary along the altitudinal gradient (Li et al. 2012). Altitude therefore indirectly defines the occurrence and distribution of insect vector species in many regions and sometimes creates buffer zones for vector-borne diseases (WHO 1975; Cox 1999). The altitudinal ranges of these climatic factors are changing with the general global climate change. These changes are likely to affect vector distribution ranges (Wettstein and Schmid 1999; Kiratani 2006). It is therefore essential to continuously monitor changes in the diversity and distribution of these vectors with the aim of preventing outbreaks of vector-borne diseases (Wettstein and Schmid 1999; Kiratani 2006). Such information can be used to determine epidemic thresholds for vector management (Bacaer and Guernaoui 2006).

The objective of this research was: (i) To determine the diversity and distribution of malaria and RVF mosquito vector species larvae along the altitudinal gradient. (ii) To evaluate habitat suitability for Malaria and Rift Valley fever vector breeding based on water quality, vegetation and presence of other organisms in a habitat.
MATERIAL AND METHODS

Description of the study area

The study area is approximately 252 km, North West of Nairobi, Kenya measuring approximately 3,500 km². It lies in an agro-pastoral zone within Baringo County, Kenya. Temperature range is between 24⁰ in the cold season and 30° degrees in the warm season. Average annual rainfall in the highland is between 1000 m and 1500 m while the low lands experience a yearly rainfall of about 600 mm. It is located between 35.602 E, 0.541N and 36.277 E, 0.723 N with elevation ranging from 800 m to 2300 m (Figure 1). This area is characterized by the presence of lakes and rivers, some of which are seasonal.

Sampling points

Sixteen sampling points were established in the study area with the help of officers from Marigat DVBDU and google earth android application. The scores were selected based on the availability of potential larval habitats and accessibility. Coordinates and elevation of each point were recorded from a handheld GPS receiver (Garmin, model etrex 10).

Elevations were divided into three classes for analysis, based on the land cover as viewed on an Arc map 3.0 imagery base map (Figure 1). They included 800 m to1300 m to represent low altitude gradient, 1301 m to 1800 m for mid-altitude and 1801 m to 2300 m for high altitude. The class range was obtained by subtracting the lowest (800 m) from the highest point (2300 m) in the study area. The difference was then divided by three. One was added to the lower limit of each class except for the first range to avoid points falling into two categories.

The sampling points were grouped in the three altitudinal classes. The Low altitude points were, Kapkuikui, Loboi, Lake 94, Nteppes, Salabani, and Kambi ya Samaki. The Middle altitude points included: Kipcherere, Kimau, Yomu, Sabor, Kabeswa, and Sabor. While the high altitude points included: Kurget, Talai, Kaplewa, Kaptimbor, Borowinin, Tandui, Sacho, Kamonol, and Sacho.

Habitat census

Potential habitats were identified within a 50 m radius from the sampling point. The 50 m range was arrived at while considering individuals undertaking the sampling exercise on foot and the minimum distance recorded in adult mosquito flight experiments (Tsuda et al. 2008; Verdonschot and Besse-Lototskaya 2014). An area was identified as a potential habitat if there was water with little to no flow (stagnant). This was because mosquitoes prefer shallow water with minimum flow/stagnant water (Norris 2004).

The habitats were classified according to their nature, based on a combination of factors. There were habitat forms such as a hoof print, swamp, water pan, dam, stream margins, spring margins, pit, lake, flood zone and marsh (Figure 2), presence or absence of vegetation, presence or absence of any other aquatic organisms apart from immature mosquitoes and water quality which was qualitatively classified as definite or turbid. The various combinations of these factors were observed and recorded during the collection of immature mosquitoes. Turbidity was estimated by dipping and collecting water with a transparent 100ml container in the habitat from down-up. Collected water was allowed to settle for 2 minutes in the bottle before checking the visibility of a three-inch white tile placed under the container. If the tile was visible, the habitat was classified as clear, and if it was not visible, the habitat was classified as turbid. In habitats too shallow for using the 100 mL container, observation was done directly in the habitat.

Sampling of mosquito larvae from aquatic habitats

Sampling for immature mosquitoes was carried out after every two weeks between 6th June 2014 and 28th August 2014. The sampling period coincided with the rainy season, and a total of five sampling sessions were completed in all selected aquatic habitats.

During sampling, immature mosquitoes were collected using 350ml WHO standard dippers at a maximum of 30 dips per habitat (Figure 3). The plastic pipette was used in extremely shallow habitats. The sampler ensured that his shadow was cast away from the habitat. This minimized chances of immature mosquitoes swimming to the bottom of the habitat. The dipper was lowered gently at an angle of 45⁰ so that collection was by displacement suction. This way, there was minimal water disturbance, increasing the probability of capturing more immature mosquitoes. Where there was dense vegetation, water was disturbed, so that larvae and pupae moved downwards. Vegetation was then cleared using the dipper. A waiting period of 3 to 4minutes would ensue before collecting the immature mosquitoes. In clumps of vegetation such as grass, the dipper was pressed gently into the plant so that water flowed in.

Figure 1. Map of Baringo County, Kenya, showing the study area and sampling points grouped into the three altitudinal classes.
Figure 2. Images of different aquatic habitats: dam margin (A); animal hoof print (B); stream bank (C); Lake Flood zone (D); water pit (E); mash (F); spring bank (G)

Figure 3. The researcher was inspecting the dipper for immature mosquitoes

After collection, the immature mosquitoes were transferred into a sealable collection cup using a plastic pipette, or directly from the habitat onto a pipette and finally into the sealable container. The collection cups were filled with water sourced from respective sampled habitats to avoid desiccation of the specimen. A pencil written label, indicating the point and date of collection were immersed into the cup before sealing and subsequent transportation to the DVBDU laboratory in Marigat.

In the laboratory, third and fourth instar larvae were identified (while still alive on a petri dish, using a dissecting microscope), and separated from second and first instar larvae. The third and fourth instar larvae were stored in labeled, sealable cups containing 80% ethanol, waiting
for identification to species level.

The first and second instar larvae were put in labeled cups, three-quarter full of water from the source habitat containing algae and loosely sealed to allow air in and out (Figure 4). They were left at room temperature (an average of 29°C during the day) to allow development to the fourth instar. Each cup contained not more than 12 larvae. An experiment conducted a week before the first collection showed that there is reduced development in instances where there were more than 12 larvae in a cup. It was also observed that development from second to fourth instar, at room temperature, took a minimum of one and a maximum of two days in a cup containing water and algae from the source habitat compared to four days when tap water was used.

Identification of larvae

Ethanol-preserved larvae were identified to species level using third and fourth instar morphological keys under guidance from experts in the Marigat DVBDU (Mark Rotich and Richard Borr). This was by observing features such as the color of the head, arrangement and shape of abdominal setae, number and type of combs, distinct hairs on the sandal, siphon index and other markings and features on the body surface as guided by the identification key (Gillies and de Meillon 1968). This was done under a dissecting microscope.

Data analysis

Species diversity analysis was performed on PAST version 2.17c. Other statistical studies were conducted on R version 3.1.1. To standardize the abundance of a species collected in each habitat, the total number of individuals collected for that species in the habitat was divided by the average number of dips in the habitat and the quotient multiplied by 30. Thirty (30) was the maximum number of dips for all habitats. Standardization ensured that figures were comparable among all habitats. Comparison of mosquitoes collected in different altitudinal classes was made using the chi-square test. Generalized linear model (GLM) was used to estimate the effect of various habitat parameters on diversity and the abundance of species. Linear correlation analysis was applied to evaluate the association between altitude and the variety and distribution of species in the study area.

RESULTS AND DISCUSSION

Diversity along the altitudinal gradient

A total of 1,536 immature mosquitoes were collected from which 10 mosquito species were identified. With respect to distribution along altitudinal gradients, 8 species (Cx. pipiens, Cx. quinquefaciatus, Cx. annulioris, Cx. poicilipes, An. pharoensis, An. coustani, An. gambiae and An. funestus) were found in altitudinal class range varying between 800 m and 1300 m (H′ = 1.462), with 7 (Cx. pipiens, Cx. quinquefaciatus, Cx. annulioris, Cx. poicilipes, An. pharoensis, An. coustani, and An. gambiae) found between 1301 m and 1800 m (H′ = 1.686) and 9 species (Cx. pipiens, Cx. quinquefaciatus, Cx. annulioris, Cx. poicilipes, An. pharoensis, Cx. annulioris, Cx. poicilipes, An. pharoensis, An. coustani, and An. gambiae) were found in the altitudinal class range between 1800 m and 2300 m (H′ = 0.9836). Of all the species identified, only seven species (Cx. pipiens, Cx. quinquefaciatus, Cx. annulioris, Cx. poicilipes, An. pharoensis, An. coustani and An. gambiae) were common in the three altitudinal zones, with An. funestus limited to lower altitudinal zone while both Cx. tigripes and Ae. taylori were found in higher altitudinal zones (1801m-2300 m) only. The Buza and Gibson evenness (eH′/S) showed that the 1301 m to 1800 m altitudinal class had higher regularity (0.77), followed by 800 m to 1300 m altitudinal level (0.53), and 1801 m to 2300 m altitudinal class (0.3) (Table 1).

Comparison of mosquitoes collected in different altitudinal classes revealed variations in the respective species counts (χ² = 127.47; p-value < 0.001). There was, however, no variation in the total number collected among the different altitudinal classes (χ² = 2.17; p-value = 0.34). Of the 1,536 immature mosquitoes collected, Cx. quinquefaciatus constituted 58.8%, dominating the species community, while An. funestus made only 0.04% of the total collection.

Distribution of mosquito species in the altitudinal ranges

The distribution of various species along the altitudinal ranges was varied (Table 1). However, most of the species showed no correlation with altitude. This is as described below.

Culex species

Four Culex species were identified in both 800 m to 1300 m and 1301 m to 1800 m altitudinal ranges with five Culex species identified in 1801 m to 2300 m altitudinal range (Table 1). Culex species identified in 800 m to 1300 m included Cx. quinquefaciatus (886.6), Cx. pipiens (206.3), Cx. annulioris (76.0) and Cx. poicilipes (18.8), while Cx. quinquefaciatus (409.0), Cx. poicilipes (126.3), Cx. pipiens (91.9) and Cx. annulioris (126.5) were identified in 1301 m to 1800 m altitudinal range. The five Culex species identified in 1801 m to 2300 m intervals included Cx. quinquefaciatus (1684.2), Cx. pipiens (175.0), Cx. annulioris (130.0), Cx. poicilipes (105.9) and Cx. tigripes (24.9).

Cx. quinquefaciatus was the most abundant mosquito species in the entire study area and the most abundant Culex species in the three altitudinal class ranges. Culex poicilipes was the least abundant in the class range between 800 m to 1300 m while Cx. annulioris and Cx. pipiens were the least abundant in the altitudinal class range between 1301 m to 1800 m. Culex tigripes was only in the altitudinal class range between 1801 m to 2300 m. It was also the least abundant in this altitudinal class range (Table 1). Further analysis showed that none of the Culex species had a significant correlation with altitude (p>0.05; Table 2).

Anopheles species

Distribution of Anopheles species in the altitudinal class ranges was, An. pharoensis (385.2), An. coustani (108.7), An. gambiae s.l (74.5) and An. funestus (18.0) between 800
m to 1300 m; *Aedes pharoensis* (205.0), An. coustani (56.3) and *An. gambiae s.l.* (39.3) between 1301 m to 1800 m; *Aedes pharoensis* (44.3), *An. gambiae s.l.* (15.0) and *An. coustani* (9.5) between 1801 m to 2300 m (Table 2).

*An. pharoensis* was the most abundant among *Anopheles* species in all altitudinal class ranges, with its population significantly correlated with altitude (r = -0.40; t = -2.50; p = 0.02; Table 2). In comparison to *Culex* and *Anopheles* species, it was the second most abundant species after *Cx. quinquefasciatus*. *An.funestus* was only found in the 800 m to 1300 m altitudinal class range with the least abundance.

**Aedes species**

* *Aedes taylori* was the only *Aedes* species present. Its distribution was not correlated with altitude (r = 0.32; t = 1.91; p = 0.07; Table 2). It was found only in the 1801 m to 2300 m altitudinal class range, with a relative abundance of 43.3.

**Effects of ecological factors on diversity and distribution of species**

Statistical analysis showed that some ecological parameters significantly affected distribution of mosquito species. Turbidity significantly affected the number of *Cx. tigripes* (Turbid; B = 2.38; t = 2.256; p = 0.0343). Habitat form significantly affected the number of *Cx. tigripes* (spring bank; B = 3.951; t = 2.403; p = 0.0251), *Cx. annulioris* (Hoof print; B = 27.2; t = -2.195; p = 0.039) and *An. pharoensis* (Marsh; B = 33.235; t = 2.319; p = 0.0301).

**Most preferred habitat for larvae**

Shannon-Weiner diversity index showed that a Riverbank, where turbidity was clear and both vegetation and other organisms were present, recorded the highest diversity of mosquito larvae species (*H’* = 1.721; Table 3). Diversity in habitats showed correlation with altitude (r = -0.34, t = -2.07, df = 32, p = 0.05; Table 2).

**Table 1.** Different mosquito species collected in different altitudinal class ranges

| Species                     | 800-1300 m | 1301-1800 m | 1801-2300 m |
|-----------------------------|-----------|------------|------------|
| *Culex pipiens*             | 206.3     | 91.9       | 175.0      |
| *Culex quinquefasciatus*    | 886.6     | 409.0      | 1684.2     |
| *Culex annulioris*          | 76.0      | 126.5      | 130.0      |
| *Culex poicilipes*          | 18.8      | 126.3      | 105.9      |
| *Cx. tigripes*              | 0.0       | 0.0        | 24.9       |
| *Anopheles pharoensis*      | 385.2     | 205.0      | 44.3       |
| *An. coustani*              | 108.7     | 56.3       | 9.5        |
| *An. gambiae*               | 74.5      | 39.3       | 15.0       |
| *An. funestus*              | 18.0      | 0.0        | 0.0        |
| *Aedes taylori*             | 0.0       | 0.0        | 43.3       |
| Total abundance             | 802.93    | 205.0      | 44.3       |

| Taxa                        | 8         | 7          | 9          |
|-----------------------------|-----------|------------|------------|
| Individuals                 | 1774.1    | 1054.3     | 2232.1     |
| D                           | 0.318     | 0.2289     | 0.5821     |
| *H’*                        | 1.462     | 1.686      | 0.9836     |
| e*H’S*                      | 0.5395    | 0.7712     | 0.2971     |

Note: *In columns are standardized numbers of mosquito larvae (Relative abundance).

Statistical analysis showed that only hoof print (B = -0.5168; t = -2.617; p = 0.0157), Water pit (B = -0.498; t = -2.345; p = 0.0284) and presence of vegetation (B = 0.597; t = 2.558; p = 0.018) significantly influenced diversity. Further analysis showed that a combination of vegetation and water quality had the greatest effect on diversity (AIC = 29.9). The most preferred habitat for larval species was therefore dictated mainly by vegetation and the level of water quality.

**Table 2.** Correlation of the effect of altitude on the distribution of different mosquito species

| Species                  | r       | T       | df  | P      |
|--------------------------|---------|---------|-----|--------|
| *Aedes taylori*          | 0.32    | 1.91    | 32  | 0.07   |
| *Anopheles coustani*     | -0.24   | -1.43   | 32  | 0.16   |
| *An. funestus*           | -0.16   | -0.92   | 32  | 0.37   |
| *An. gambiae s.l.*       | -0.18   | -1.01   | 32  | 0.32   |
| *An. pharoensis*         | -0.40   | -2.50   | 32  | 0.02   |
| *Culex annulioris*       | 0.05    | 0.31    | 32  | 0.76   |
| *Cx. pipiens*            | 0.04    | 0.22    | 32  | 0.83   |
| *Cx. poicilipes*         | 0.21    | 1.23    | 32  | 0.23   |
| *Cx. quinquefasciatus*   | 0.26    | 1.49    | 32  | 0.14   |
| *Cx. tigripes*           | 0.39    | 2.39    | 32  | 0.23   |
| Diversity in habitats    | -0.34   | -2.07   | 32  | 0.05   |

**Table 3.** Habitat species diversity

| Altitude | Habitat form | Water quality | Vegetation | Other organisms of species | Number of species |
|----------|--------------|---------------|------------|---------------------------|------------------|
| 1334     | Riverbank    | Present       | Present    | Present                   | 5                |
| 1457.79  | Dammargin    | Turbid        | Present    | Present                   | 5                |
| 1450.17  | Springbank   | Clear         | Present    | Present                   | 5                |
| 1019.81  | Marsh        | Clear         | Present    | Present                   | 5                |
| 1457.79  | Dammargin    | Clear         | Present    | Present                   | 5                |
| 1925     | Dammargin    | Clear         | Present    | Present                   | 4                |
| 1450.17  | Springbank   | Clear         | Present    | Present                   | 4                |
| 982.93   | Lakemargin   | Clear         | Present    | Present                   | 4                |
| 1323     | Riverbank    | Clear         | Present    | Present                   | 5                |
| 987.2    | Floodzone    | Turbid        | Present    | Present                   | 3                |
| 982.93   | Hoofprint    | Clear         | Present    | Present                   | 3                |
| 1019.81  | Marsh        | Turbid        | Present    | Present                   | 4                |
| 987.2    | Floodzone    | Clear         | Absent     | Absent                    | 3                |
| 983.24   | Lakemargin   | Clear         | Present    | Present                   | 3                |
| 2212     | Springbank   | Clear         | Present    | Present                   | 6                |
| 1015.24  | Marsh        | Clear         | Present    | Present                   | 5                |
| 983.24   | Lakemargin   | Clear         | Present    | Present                   | 4                |
| 987.2    | Floodzone    | Clear         | Present    | Present                   | 3                |
| 1457.79  | Dammargin    | Absent        | Absent     | Absent                    | 2                |
| 987.2    | Floodzone    | Turbid        | Present    | Present                   | 2                |
| 2140     | Waterpit     | Clear         | Present    | Present                   | 2                |
| 999.7    | Hoofprint    | Clear         | Present    | Present                   | 2                |
| 999.7    | Waterpit     | Clear         | Present    | Absent                    | 2                |
| 2177     | Waterpan     | Clear         | Present    | Absent                    | 2                |
| 1837     | Dammargin    | Clear         | Present    | Present                   | 2                |
| 2177     | Waterpan     | Turbid        | Present    | Absent                    | 3                |
| 2140     | Waterpit     | Turbid        | Present    | Present                   | 4                |
| 999.7    | Hoofprint    | Clear         | Present    | Present                   | 2                |
| 2212     | Springbank   | Absent        | Absent     | Absent                    | 1                |
| 2179     | Waterpit     | Absent        | Absent     | Absent                    | 1                |
| 2179     | Waterpit     | Absent        | Absent     | Absent                    | 1                |
| 2179     | Waterpit     | Absent        | Absent     | Absent                    | 1                |
| 999.7    | Hoofprint    | Absent        | Present    | Absent                    | 1                |
| 997.2    | Floodzone    | Turbid        | Absent     | Absent                    | 1                |

DANCAN – Vectors of malaria and rift valley fever along altitudinal gradient in Kenya 29
Discussion

The only *Aedes* species identified was *Ae. taylori* in the altitudinal range between 1801 m to 2300 m. This species has been implicated as a vector of yellow fever in sylvatic transmission. Its ability to feed on monkeys and humans enables it to spread the yellow fever virus from monkeys to human beings (Digoutte et al. 1999). Primary infections of RVF are a result of floodwater *Aedes* species which are considered as reservoir hosts of RVF virus due to trans-ovarian transmission and ability of the eggs to diapause in the soil for months or years until there is flooding (Sang et al. 2010). Flood water *Aedes* species in Kenya include *Ae. mintoshi*, *Ae. ocharacieus*, *Ae. sudanensis*, and *Ae. circumluteolus* (Lutomiah et al. 2013).

None of these species were identified in the entire study area within the study period. The results, therefore, indicate that there was no risk of RVF first outbreak based on the identified vectors in the study area during the study period. This was consistent with Baringo county vector-borne disease unit (VBDU) data and public health records. They indicated no cases of RVF were reported between January 2013 and September 2014 within the study region.

The RVF virus has previously been isolated in all the three genera identified in the study area (Sang et al. 2010). Many species of mosquitoes and sandflies are susceptible to RVF if they feed on an infected host and can cause secondary transmission of the virus. The wide range of such secondary vectors is what causes sudden epidemics of the virus after primary infection by the flood water *Aedes* species (Linthicum et al. 1985). This indicated that, although there were no primary vector larvae species identified in the study area, in case of entry of infected individuals such as cattle into the region, there would be a possible epidemic especially if this was in the rainy season as mosquito species reach their peak abundances during such seasons (Uyi 2013).

Among the identified 10 species, five were *Culex* mosquito species; *Cx quinquefaciatus*, *Cx. pipiens*, *Cx. annulioris*, *Cx. poicilipes* and *Cx. tigripes*. Apart from being secondary vectors of RVF, *anop* species have been implicated as vectors of various other arbovirus diseases. An example of such a disease is the West Nile Virus. The west Nile Virus is transmitted by *Culex* species, from birds to humans and other mammals. This is a result of their ability to feed on both mammals and birds (Molei et al. 2006). Evidence of the West Nile virus transmission in Kenya was found in mosquitoes collected in various parts including the former Rift valley province which Baringo, currently Baringo County, was part of (LaBeaud et al. 2011). None of the *Culex* species showed a significant correlation to altitude. This implied that in case of emergence of RVF, West Nile Virus or any other disease spread by the *Culex* species, whose distribution was not limited by altitude, the disease might spread rapidly in the entire county if rapid interventions are not initiated.

*Culex quinquefaciatus* was the most abundant species in the study area, and *Cx tigripes* was the least abundant. *Culex quinquefaciatus*, apart from being among secondary vectors of RVF in epidemics in Kenya (Sang et al. 2010), it is also the primary vector of urban lymphatic filariasis, caused by the nematode *Wuchereria bancrofti* (Bockarie et al. 2009). However, there are no cases of vector-transmitted filariasis in Baringo County. Any examples that come in are from the coastal regions of Kenya. Mosquito species in the area are not able to transmit the disease (unpublished data, Baringo County, VBDU). *Culex tigripes* is a predator of other mosquito larvae and can be used as larval biological control (Appawa et al. 2000). With the increase in highland malaria all over Kenya and considering it was in the high altitude regions, it can be exploited as a measure of reducing highland malaria transmission.

Like *Cx. quinquefaciatus* and *Cx. tigripes*, the other three *Culex* species, *Cx. pipiens*, *Cx. annulioris* and *Cx. poicilipes* did not show any significant correlation with altitude. This is an indication that any diseases they transmit can be spread both in the highlands and the lowlands leading to infections in the entire region. *Culex pipiens* was implicated as the primary vector maintaining the RVF epidemic in Egypt from 1971 to 1978 (Hooogstraal et al. 1979). The laboratory test of *Cx. pipiens* strains have also shown that apart from being susceptible to RVF virus, they are also vulnerable to West Nile Virus (Amraoui et al. 2012). It is also a primary vector of the Ndumu Virus (NDUV) as reported in a study done in Garissa, Kenya, where evidence of trans-ovarian transmission of the virus was recorded (Lutomiah et al. 2014). Studies in Senegal indicated that *Cx. poicilipes* was the main RVF virus vector after the 1998 outbreak in Mauritania (Diallo et al. 2000). RVF viruses were isolated from *Cx. annulioris* species in the 2007/2008 epidemic in Kenya (Sang et al. 2010). These are further indications that all the *Culex* species identified in the study area are secondary vectors of RVF and therefore the fact that elevation does not limit them indicates that all regions of Baringo County have a potential risk of RVF secondary outbreaks.

Among the four *Anopheles* species identified in the study area, only *An. pharoensis* showed a significant correlation with altitude. However, between the altitudinal classes, the least abundances of *Anopheles* species were in the high altitude class (1801-2300 m). *Anopheles pharoensis* was the most abundant *Anopheles* species and second most abundant after *Cx. quinquefaciatus* amongst all species identified in the study area. This is contrary to what a study in 2011 established, where a sibling species of *An. gambiae s.l*, and *An. arabiensis* was the most abundant (Mala et al. 2011). *Anopheles pharoensis* has been documented as an efficient malaria vector in Senegal (Carrara et al. 1990). It might also be an efficient vector in Baringo County considering the many cases of malaria, which were higher during the study period (Unpublished data, Baringo county public health records). Studies on its biting habits in Kapkuikui village, Baringo County indicated that it bites more often outdoor than indoor and is exophilic (Aniedu 1993). This might be the reason for its success since interventions in Baringo County mostly involve the use of insecticide-treated bed nets and pyrethrum spraying inside houses. These affect indoor biters. *Anopheles funestus* and *An. gambiae s.l* are documented as endophilic and prefer biting indoors than
outdoors (Aniedu 1993). This might explain their low larval abundances compared to An. pharoensis. Anopheles funestus larvae were the least abundant amongst Anopheles species. They were identified only in the low altitude region (800 m to 1300 m). This is consistent with findings in a study done in 2011 within the low altitude region where it was the least abundant species (Mala et al. 2011). Anopheles costanti had a higher abundance than An. funestus and An. gambiae, but lower than An. pharoensis.

Individual species responded to different ecological parameters in the same habitat differently while others were not affected by any of the recorded parameters. Results on Culex species are consistent with findings in a study carried out in villages within Mwea, Kenya, where Culex species responded differently to various ecological parameters in habitats (Muturi et al. 2007). In this study, only Cx. annulioris and Cx. tigripes responded to the recorded habitat parameters; hoof print habitat form for Cx. annulioris, spring bank habitats form and turbidity for Cx. tigripes. Vegetation and turbidity in habitats had the most significant influence on diversity.

However, diversity in habitats had a negative correlation with altitude, indicating that habitat diversity reduced as height increased. On the interaction between species in a habitat, none of the Culex species showed any significant interactions. However, there were significant interactions between An. funestus and An. costanti. Anopheles pharoensis showed substantial interactions with An. costanti in the habitats.

In conclusion, the study hypothesis predicted that immature stages of malaria and RVF vector species vary amongst habitats along the altitudinal gradient. However, the results show only the distribution of An. pharoensis had a negative association with altitude. The implication of this is a need for continuous monitoring of vector species to avoid malaria and RVF outbreaks that would likely affect highlands and lowlands, assuming the vector competence of adult mosquitoes found in both regions is similar. During monitoring, habitats that have clear water with vegetation would be the most probable culprit for larvae breeding.

REFFERENCE

Amraoui F, Krida G, Bouattour A, Rhim A, Daaboub J, Harrat Z, Failloux AB. 2012. Culex pipiens, an experimental efficient vector of West Nile and Rift Valley fever viruses in the Maghreb region. PlosOne 7: e36757. DOI: 10.1371/journal.pone.0036757
Aniedu I. 1997. Dynamics of malaria transmission near two permanent breeding sites in Baringo district, Kenya. Indian J Med Res 105: 206-211.
Appawu MA, Dadzie SK, Quartey SQ. 2000. Studies on the feeding behaviour of larvae of the predaceous mosquito Culex (Lutzio) tigripes Grandpre and Chamyoo (Diptera: Culicidae). Int J Trop Insect Sci 20: 245-250.
Bacak N, Guernaoui S. 2006. The epidemic threshold of vector-borne diseases with seasonality. J Math Biol 53: 421-436
Bockarie MJ, Pedersen EM, White GB, Michael E. 2009. Role of vector control in the global program to eliminate lymphatic filariasis. Ann Rev Entomol 54: 469-487.
Carrara GC, Petrarca V, Niang M, Coluzzi M. 1990. Anopheles pharoensis and transmission of Plasmodium falciparum in the Senegal River delta, West Africa. Med Vet Entomol 4: 421-424.
Cox J. 1999. Mapping Malaria Risk in the Highlands of Africa. MARA/Durban. London School of Hygiene and Tropical Medicine, London.
Dullo M, Lochouarn L, Ba K, Sall AA,ondo M, Girault L, Mathiot C. 2000. First isolation of the Rift Valley fever virus from Culex pipiens (Diptera: Culicidae) in nature. Amer J Trop Med Hygiene 62: 702-704.
Digojte JP. 1999. Present status of an arbovirus infection: yellow fever, its natural history of hemorrhagic fever, Rift Valley fever. Bulletin de la Societe de Pathologie Exotique 92: 343-348.
El Vilyal AE, Aoroa M, Butterworth MK, Jamagin W, Comrie AC. 2013. Climate, environment and disease: The case of Rift Valley fever. Progr Phys Geogr 37 (2): 259-269.
Gillies MT, de Meillon B. 1968. The anophelines of Africa, south of the Sahara. Johannesburg: The South African Institute for Medical Research 220-330
Githmeko AK, Lindsay SW, Confalonieri UE, Patz JA. 2000. Climate change and vector-borne diseases: a regional analysis. Bulletin of World Health Organization 78 n.9 Geneva Jan.
Hooogstraal H, Meegan JM, Khalil GM, Adham FK. 1979. The Rift Valley fever epizootic in Egypt 1977-1978. 2. Ecological and entomological studies. Trans R Soc Trop Med Hygiene 73: 624-629.
Kenya Medical Research Institute. 2014. Kenya malaria fact sheet. Kenya medical research institute, http://www.kemri.org/index.php/helpdesk/search/diseases-a-conditions/29-malaria/113-kenya-malaria-fact-sheet.
Kiritani K. 2006. Predicting impacts of global warming on population dynamics and distribution of arthropods in Japan. Population Ecology 48: 5-12.
LaBeaud AD, Sutherland LJ, Muinuri S, Muchiri EM, Gray LR, Zimmerman PA, King CH. 2011. Arbovirus prevalence in mosquitoes, Kenya. Emerg Infect Dis 17: 233-241.
Li Z, He Y, Winfred H, Wang X, Zhang W, Cao W, Du J, Xin H, Chang L. 2012. Altitude dependency of trends of daily climate extremes in southwestern China, 1961–2008. J geographical science 22: 416-430.
Linthicum KJ, Davies FG, Kairo A, Bailey CL. 1985. Rift Valley Fever virus (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. J Hygiene 95: 197-209.
Lutomiah J, Bast J, Clark J, Richardson J, Yalwala S, Oulio D, Sang R. 2013. Abundance, diversity, and distribution of mosquito vectors in selected ecological regions of Kenya: public health implications. J Vector Ecol 38: 134-142.
Lutonmiah J, Onsug J, Linthicum KJ, Sang R. 2014. Natural Vertical Transmission of Ntunu Virus in Culex pipiens (Diptera: Culicidae) Mosquitoes Collected as Larvae. J Med Entomol 51: 1091-1095.
Mala AO, Irungu LW, Shilul JI, Muturi EJ, Mbogo CC, Njagi JK, Githure JJ. 2011. Dry season ecology of Anopheles gambiae complex mosquitoes at larval habitats in two traditionally semi-arid villages in Baringo, Kenya. Parasit Vect 4: 1-11.
Molaei G, Andreadis TG, Armstrong PM, Anderson JF, Vossbrinck CR. 2006. Host feeding patterns of Culex mosquitoes and West Nile virus transmission, northeastern United States. Emerg Infect Dis 12: 468-474.
Muturi EJ, Shilul JI, Gu W, Jacob BG, Githure JJ, Novak RJ. 2007. Larval habitat dynamics and diversity of Culex mosquitoes in rice agro-ecosystem in Mwea, Kenya. Amer J Trop Med Hygiene 76: 95-102.
Norris DE. 2004. Mosquito-borne diseases as a consequence of land use change. EcoHealth 1: 19-24.
Sang R, Kioko E, Lutomiah J, Warigia M, Ochieng C, O’Quinn M, Richardson J. 2010. Rift Valley Fever Virus Epidemic in Kenya, 2006/2007: The Entomologic Investigations. Amer J Trop Med Hygiene 83: 28–37.
Swanepoel R, Pawsaka JT. 2011. Rift valley fever. Oxford textbook of Zoonoses: Biology, Clinical Practice, and Public Health. Oxford Univ Press, London.
Tsuda Y, Komagata O, Kasai S, Hayashi T, Nihei N, Saito K, Kobayashi M. 2008. A mark-release-recapture study on dispersal and flight distance of Culex pipiens pallens in an urban area of Japan. J Amer Mosquito Control Assoc 24:339-343.
Uyi COO. 2013. Temporal Distribution of and Habitat Diversification by Some Mosquitoes (Diptera: Culicidae) Species in Benin City, Nigeria. J Entomol 10: 13-23.
Verdonschot PF, Besse-Lootskaykya AA. 2014. Flight distance of mosquitoes (Culicidae): A metadata analysis to support the management of barrier zones around rewetted and newly constructed wetlands. Limnol Ecol Manag Inland Waters 45: 69-79.
Wettstein W, Schmid B. 1999. Conservation of arthropod diversity in montane wetland; effects of altitude, habitat quality and habitat fragmentation on butterflies and grasshoppers. J Appl Ecol 36: 363-373.

Woods CW, Karpati AM, Grein T, McCarthy N, Gaturuka P, Muchiri E, World Health Organization Hemorrhagic Fever Task Force. 2002. An outbreak of Rift Valley fever in northeastern Kenya. 1997-98. Emerg Infect Dis 8: 138-144.

World Health Organization. 1975. Manual on practical entomology in malaria. WHO, Geneva.

World Health Organization. 2007. Rift Valley Fever in Kenya, Somalia and the United Republic of Tanzania. World Health Organization Global alert and response. WHO, Geneva.

World Health Organization. 2013a. Malaria entomology and vector control. World Health Organization, Geneva.

World Health Organization. 2013b. Malaria report. World Health Organization, Geneva.
Influence of human activity on diversity and abundance of insects in three wetland environments in Ghana

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Abstract. Mensah BA, Kyerematen R, Annang T, Adu-Acheampong S. 2018. Influence of human activity on diversity and abundance of insects in three wetland environments in Ghana. Bonorowo Wetlands 2: 33-41. The Wetland environment is unique with unique biota that includes insects. Insects serve as indicators of environmental health, nevertheless, the recent state of human encroachment on wetlands is likely to affect its unique biotic composition, and this phenomenon poses a threat to the wetland environment. The physical and chemical quality of studied habitats in this research provided background information for comparison against the established quality standard of the wetland environment. The study involved reconnaissance surveys, insect trapping and social surveys on the impact of anthropogenic activities on insect diversity and abundance in and around the wetland environment. Twenty-two insect orders belonging to 112 families were sampled from different sites along the Sakumono, Kpeshie, and Muni-Pomadze wetlands. Species diversity and abundance were significantly different among the various locations with the most diverse being Kpeshie. Water within wetlands in Kpeshie was the most polluted although it had a positive correlation with insect diversity and abundance. Results of a survey of selected communities showed that majority of the residents had a low level of education with less appreciation of issues involving the environment including pollution. Majority of people within the surveyed communities were unable to access decent toilet facilities and publicly demarcated waste disposal sites. There was no coordinated and concerted effort to manage these three wetlands two of which are designated Ramsar sites. Activities such as farming, discharge of domestic garbage, improper fishing practices, improper disposal of industrial and human waste are increasing the pollution risk of these wetland environments.

Keywords: Abundance, diversity, insect, lagoon, wetland

INTRODUCTION

Wetlands are highly diverse habitats and are known to be among the earth’s most productive ecosystems (Barbier et al. 1997). Wetlands can be classified based on their (i) components (biotic and abiotic features), (ii) functions (the interactions between the components such as nutrient cycling), and (iii) attributes such as species diversities. These characteristics of wetlands support human existence and their related economic activities. “Wetlands” is an elastic term which includes large variety of landforms. Some cold climate wetlands are unique and thus have no tropical equivalence, and vice versa. Tundras and mangroves, for instance, are unique to the temperate and tropics respectively.

Wetland resources comprise of the water, land, soils, plants, and animals, which may be exploited for subsistence, income, and employment. While wetland services such as maintenance of hydrological and biogeochemical cycles, act as management schemes for silt and other materials, erosion control plays a dominant role in maintaining the general ecological balance. For instance, it has been reported that rivers and wetlands around Lake Victoria act as natural purifiers (Scheren et al. 2000). Besides supplying local communities with resources for subsistence, wetlands support distant communities with ecological services such as flood and water flow regulation and drought alleviation, ground-water recharge, water quality protection and purification, drinking water supply and storage, erosion and sediment control, wastewater treatment, carbon retention and climate modification (Seyam et al. 2001). The New Partnership for Africa’s Development (NEPAD) has identified six sectoral priorities that include the Environment Initiative in Africa which included wetland conservation which is recognized as one of the eight sub-themes for priority intervention (Anon 2001).

Insects are vital for ecosystems functioning (Samways et al. 2010). They can inhabit all conceivable habitats from the pole to the equator and occupy all trophic niches more than the level of primary producers (Resh and Carde 2003). Insects are known to be the most abundant and diverse organisms present in most environments. Insects may be used as indicators of environmental quality (Kyerematen et al. 2014 a, b; 2018 a, b; Acquah Lamptey et al. 2013 a, b, Adu-Acheampong et al. 2016) due to their short life cycles and sensitivity to perturbations. In most terrestrial ecosystems, insects are the dominant herbivores. They may significantly influence the plant community as well as reflect the variety of plant resources available to them (Barbour et al. 1998; Groves 2002). Insects in wetlands are abundant and diverse because wetlands have too shallow
water depths to support the lives of many fish species and thus exerting little or no pressure on insect species which act as fish prey. The absence of many fish and other insect predators within wetlands create convenient habitats which enable insects to survive and persist especially in swampy areas.

Insects are for the large part, responsible for the breakdown of organic material such as plant, animal and animal remains, the elimination of animal waste, the aeration of the soil and the vastly important task of plant pollination. They are an essential food source for many fishes, birds, amphibians, and reptiles. Furthermore, in some parts of the world, they also constitute a significant portion of the human diet. Rare insects are sometimes used as indicators of endangered mammal species. In spite of that, insects and such related indicator species are given little attention despite their importance in the overall ecological balance (Constanza et al. 1997).

In this study, we investigated insect diversity of wetlands in three wetland environments in relations to human activities. We also investigated the impact of anthropogenic activities on wetland within the study areas using the relationship between pollution and insect diversity in these wetlands. The aim was to use the presence or absence and abundance of key insect species as proxy measures for degradation or otherwise of the wetland environments within the study areas. We hypothesize that the presence (diversity and abundance) of some insects groups is dictated by pollution within these wetlands.

MATERIALS AND METHODS

Study areas

Winneba

Winneba is located on the south coast (56 km west of Accra and 140 km east of Cape Coast). The Muni-Pomadze wetland in Winneba in the Central Region of Ghana, (Figure 1.A) is one of five internationally-recognized coastal wetlands (Ramsar sites) in Ghana under the Convention on Wetlands of International Importance (Ramsar Convention), thanks to its importance as a breeding and nesting site for migratory and resident waterbirds, insects, and terrestrial vertebrates (Collar et al. 1994; Ryan and Attuquayefio 2000; Kyerematen et al. 2014a).

The wetland is particularly vital to the local Effutu people, serving as their traditional hunting grounds, especially during their annual "Aboakyer" Festival. The swamp falls within the Coastal Savanna Vegetation Zone of Ghana, with a characteristic bimodal rainfall distribution and a low mean annual rainfall of about 854 mm. According to Gordon and Cobbblah (2000), the dominant rainy season occurs from March/April to July/August with a peak in June, while the minor season runs from September to November. The dominant dry season runs from December to March and the minor dry season from August to September. Mean annual temperature ranges from 24°C in August to 29°C in March with a relative humidity range of 75-80% (Gordon and Cobbblah 2000). The site selected for the survey lies within the boundaries of the proposed Muni-Pomadze Ramsar site. The principal sampling area was located near Mankoadze, a fishing village west of Winneba.

In recent times, the previously diverse fauna of this area including mongooses, have dwindled, with some of the animals presumed locally extinct or rare (Ryan and Attuquayefio 2000). Current evidence suggests that the degradation of the wetland could be attributable primarily to neglect and unsustainable human activities such as bushfires, farming, hunting, fuelwood harvesting and estate development (Ntiamo-Baidu and Gordon 1991; Ryan and Ntiamo-Baidu 1998; Kyerematen et al. 2014a).

Sakumo Lagoon

The Sakumo Lagoon is situated on the eastern part of Accra along the Accra-Tema coastal road 3 km west of Tema (Figure 1.B). The lagoon is located within latitudes 5° 36.5” N and 5° 38.5” N and between the longitudes 1° 30’ W and 2° 30’ W. The district stretches from Madina to Oyarifa on the west and the Aburi highlands in the north. It is bounded by an approximate north-south line on the east, which also defines the western boundary of Tema (Biney 1995b). The surface area is 2.7 km² and its catchment area covers a total area of 350 km² although the active catchment area is 127 km² because of damming of the streams leading towards the lagoon (Tumbulto and Bannerman 1995).

There are two rainy seasons with the major season starting in March and peaking in mid-July and the minor season beginning in mid-August and ending in October. The average annual rainfall is about 753 mm, and relative humidity varies from an average of 65% in mid-afternoon to 95% at night. The mean monthly temperatures range from a minimum of 24.7°C in August to a maximum of 28.1°C in March. The lagoon and its neighboring wetlands have been labeled as one of the five coastal Ramsar sites in Ghana (Kwei 1974).

Sakumo Lagoon is still a vast birding destination despite its position in the heart of a sprawling metropolis. Extending about 20 km east of Accra and covering up to 350 ha, Sakumo Lagoon is ideally situated for birding from the city in either the morning or afternoon. The main attraction at Sakumo is the open shallow estuary and flooded reedbeds which between September and April can support thousands of waders and an impressive list of estuarine birds. The surrounding savanna also hosts many species from dry country species and birds of prey. A couple of hours birding in the morning or afternoon at Sakumo between October and April should produce upwards of 80 species (Ryan 2005, Ntiamo-Baidu and Gordon 1991). It was labeled as a Ramsar site on the 14th of August 1992, and it is managed by the Wildlife Division of the Forestry Commission on behalf of the state.
Figure 1. Map of the study area in Muni-Pomadze Lagoon (Winneba) (A), Kpeshie Lagoon (B), Sakumono Lagoon (C) and Ghana
**Kpeshie Lagoon**

The Kpeshie lagoon catchment area lies between latitude 5° 33'0" N and 5° 36'20" N and stretches between longitude 0° 9'30" W and 0° 7'10" W (Figure 1.C). The catchment area occupies almost 47.391551 ha. The Kpeshie lagoon is less than 1km² in surface area, and it is situated along the outskirts of La, a peri-urban township. The Municipal Assembly share boundaries with the following Sub Metros: Osu Clottey towards the east, Ayawaso towards the north, and Teshie to the west (Kpanja 2006).

**Methods**

**Sampling points**

Sampling points capture the main activities carried out along the stretch of the lagoons which would affect the water quality and insect diversity.

**Sampling design and technique**

The following trapping techniques were used to capture insects: malaise traps, yellow pan traps, light traps, fruit-baited charaxes traps, pitfall traps, flight interception traps, sweep nets and aerial nets. A regular, perpendicular walk was undertaken from predetermined sites to all the selected locations along the lagoons. To ensure that the traps were proportionally spaced, a meter tape was used to measure the distance between them. The smallest intersite distance was 50m with the largest being 250m. Where necessary a hoe and machete were utilized to cut through grass or mangrove to gain access. Sampling was done monthly during the rainy season (April to June) and the dry season (December to February) and the temperature was recorded during these seasons.

**Malaise trap**

This trap has been designed to collect flying insects. This rectangular tent-like trap made of black nylon netting directs the flying insects into a collecting bottle containing 70% alcohol at the top end of one side. Insects were collected after 3-5 days for subsequent identification.

**Pitfall trap**

This is used for trapping ground inhabiting insects and is a straight-sided container that is sunk level with the surface of the neighboring substratum. Ten traps were set at 20m intervals along the 200m transect in each area. Each trap contained a soapy solution to break the surface tension so that trapped insects would not be able to fly or crawl out. Trapped insects were collected after 3-5 days and emptied into a container containing 70% alcohol for subsequent identification.

**Yellow pan trap**

Yellow pan traps collect insects that are attracted to the yellow color filled with soapy water. Ten traps were set at 20m intervals along the 200m transect in each area. Trapped insects were collected after 3-5 days and emptied into plastic bottles containing 70% alcohol for subsequent identification.

**Flight interception trap**

This trap is commonly used to intercept flying insects which are not likely to be drawn to baits or light and is assembled of brightly colored netting. Intercepted insects fall into the trays at the bottom that contain a killing agent. One trap was set in each area. Trapped insects were collected after 3-5 days and emptied into bags containing 70% alcohol for subsequent identification.

**Charaxes trap**

This trap is made up of a net with a rectangular cross-section with a string attached to the four corners at the closed top and a flat wooden board connected at the open end. Bait made up of mashed rotten banana mixed with palm wine was placed on the board. Alcohol-loving insects are mostly trapped by this method. Individual traps within areas were separated from each other by at least 50m and by no more than 250m (Oduro and Aduse-Poku 2005). Standard field handling of specimens captured from charaxes traps consisted of firmly squeezing the thorax to disable the sample (Oduro and Aduse-Poku 2005).

**Sweep net**

The sweep net consists of a circular metallic rim with a cloth attached to form a sac with the rim as the opening with a wooden handle attached to the rim. It was swung through the vegetation with the alternating forehand and backhand strokes about 10 times and the content carefully emptied into a killing jar. The catches were later transferred into a bag containing 70% alcohol for subsequent identification.

**Aerial net**

The aerial net consisted of a metallic rim with a wooden handle and a fine mesh forming a sack. Swarming butterflies, dragonflies and moths were spotted and collected. The butterflies caught were placed in glassine envelopes with wings folded together. This technique prevented the insects from losing their scales, a feature very vital for identification. The other insects were transferred into killing jars containing ethyl acetate and kept in glassine envelopes for later identification.

**Visual observation and direct counts**

Visual counts were done whenever an insect was spotted that was out of reach to be collected or trapped. At each site, random walk sampling was used for a minimum of two hours to sample each site twice daily. This was done under sunny conditions mostly between the hours of 8:00 hours GMT and 16:00 hours GMT. The butterflies were identified by their wing patterns and colors as well as flight patterns.

**Social survey**

**Sampling technique**

This study employed a purposive sampling technique, the non-probability sampling technique.
Questionnaire administration

Questionnaires were administered in the major towns/settlements where water samples were collected in a purposive sampling and non-probability technique. A total of 280 questionnaires were administered in Winneba, Sakumo, and Kpeshie. An effort was made to interview women and men equally in each locality.

Interview

Interviews were conducted by interacting with some of the locals in sensitive areas such as the small-scale industries and the lagoon sites.

Non-participatory observations

Non-participatory observations were also undertaken to enable the interviewer to generate initial information to complement the data obtained from respondents.

Sorting and species identification

All insects sampled were either placed in containers containing 70% alcohol or envelopes and labeled for further identification. All insects were identified to specific level with reference to Museum collections in the Biodiversity Museum of the Department of Animal Biology and Conservation Science, University of Ghana, Legon, Scholtz and Holm (2005), Carter et al. (1992), Gullan and Craston (2005), and Boorman (1981).

Statistical analyses

Several statistical analyses were performed using SPSS (Vol.16.0) to determine if some of these environmental factors affected insect diversity within the wetlands. Data obtained from the traps at a given sampling point on a specific date were pooled to generate a single sample for each site-data combination. Data from all the traps were combined to get total insect diversity per study site and sampling period. Simpson's Index (D) Shannon-Weiner Index (H), Margalef index and the Pielou’s Evenness index (“J”) were computed for measuring insect diversity.

A one-way ANOVA of insect diversity between groups of wetlands was performed to test whether there was a difference in insect diversity among the three sites (Muni-Pomadze, Sakomo lagoon, and Kpeshie lagoon). Simpson/Shannon diversity indices and Margalef richness index were calculated. Nonparametric richness estimators were applied to estimate total species richness at a site: ACE (Abundance-based coverage estimator), ICE (Incidence-based coverage estimator), Chao1 (Abundance-based coverage estimator), Chao2 (Incidence-based coverage estimator), Jack1 (First-order jackknife estimator) and jack2 (Second-order jackknife estimator).

RESULTS AND DISCUSSION

Relative abundance of insects

As many as 5,541 individual insects were recorded from all the three sites combined. Muni-Pomadze recorded 1,883, Sakumo wetlands recorded 1,550 and Kpeshie recorded 2,128. Four hundred and twenty-nine species of insects, belonging to 22 orders and 112 families were collected from all three sites. Insects belonging to the orders Hymenoptera, Diptera, Hemiptera, and Coleoptera were the most abundant and diverse in all three areas (Table 1). Hymenoptera and Diptera had the highest relative abundances of 34% and 30% respectively during the dry season. Meanwhile, Hymenoptera and Hemiptera were dominant with relative abundances of 25% and 19% respectively for the wet season.

There was no significant difference between Sakumo and Kpeshie sites, but Muni-Pomadze was significantly different from the two. The average of at least two groups of the analysis differed significantly (p>0.05). The abundance of insects did not vary much between Kpeshie and Sakumo, but that of Muni-Pomadze was relatively less than both Kpeshie and Sakumo, however, there was a significant difference in the relative abundance of insects sampled during the dry and the wet season. At all sampling sites, the number of individual insects collected in the wet season was higher than the dry season (Table 1). Hymenoptera was the richest order in both seasons at Sakumo with a relative abundance of 29.9% for the wet season and 9.0% for the dry season. Kpeshie recorded an overall relative abundance of 88% in the wet season and 12% in the dry season with Muni-Pomadze recording a total relative abundance of 84% in the wet season and 15.6% in the dry season.

Species richness and diversity indices

Species richness in terms of Margalef and Pielou indices were higher in the wet seasons at all three sites than the dry seasons, but the Shannon Weiner and Simpsons diversity indices were somewhat higher in the dry season than that of the wet season (Table 2).

Observed species richness

Table 3 shows that the observed species richness (Sobs) was higher at Kpeshie than the rest of the sites. The species accumulation curves were approaching an asymptote, an indication that species saturation had been reached and sampling efforts were adequate.

Social survey: Respondent information

Sex

The questionnaires were administered in Sakumo, La Trade Fair Area (Kpeshie) and Mankoadze (Muni-Pomadze) in Winneba. The distribution of the polls was done to ensure that there were an almost equal number of men (137) and women (143). The population tested in each of these locations was categorized according to the level of activities carried out by residents in these catchment areas. There were 100 respondents each from Sakumo and Mankoadze constituting 71.4% of the total respondents for each of the two sites. The rest of the respondents were from the Trade Fair Area (Kpeshie), representing 28.6% of the 280 people sampled for the study. Forty-six percent and 42% of respondents from Trade Fair and Mankoadze respectively disposed of their refuse indiscriminately (Figure 2).
Table 1. Relative abundance of individual insects captured by order from the three wetlands

| Order             | Sakumono | Kpeshie | Muni-Pomadze | Relative abundance % |
|-------------------|----------|---------|--------------|----------------------|
| Coleoptera        | 93       | 218     | 372          | 12                   |
| Diptera           | 256      | 524     | 299          | 20                   |
| Hymenoptera       | 595      | 518     | 400          | 27                   |
| Hemiptera         | 188      | 415     | 214          | 15                   |
| Lepidoptera       | 105      | 112     | 145          | 7                    |
| Orthoptera        | 89       | 69      | 96           | 4.6                  |
| Dictyoptera       | 33       | 36      | 36           | 2                    |
| Collembola        | 51       | 54      | 79           | 3                    |
| Dermaptera        | 3        | 3       | 14           | 0.4                  |
| Odonata           | 15       | 14      | 33           | 1                    |
| Ephemeroptera     | 6        | 6       | 17           | 0.5                  |
| Mallophaga        | 0        | 0       | 9            | 0.1                  |
| Embioptera        | 3        | 0       | 14           | 0.3                  |
| Psocoptera        | 26       | 17      | 43           | 1.5                  |
| Neuroptera        | 18       | 18      | 16           | 0.9                  |
| Trichoptera       | 6        | 23      | 26           | 0.9                  |
| Thysanoptera      | 18       | 58      | 43           | 2.2                  |
| Mecoptera         | 0        | 4       | 5            | 0.1                  |
| Isoptera          | 10       | 4       | 5            | 0.7                  |
| Anoplura          | 0        | 4       | 0            | 0.07                 |
| Homoptera         | 14       | 4       | 14           | 0.6                  |
| Plecoptera        | 1        | 0       | 3            | 0.07                 |
| Total individual number (N) | 1530         | 2118     | 1883         | 100               |

Table 2. Diversity and richness indices for each season in and around each lagoon

| Sites             | Simpson (I/D) | Shannon-Weiner (H) | Margalef (D) | Pielou (J) |
|-------------------|---------------|--------------------|--------------|------------|
|                   | Wet           | Dry                | Wet          | Dry        | Wet | Dry |
| Sakumono          | 10.8          | 13.10              | 4.15         | 3.57       | 131.9 | 58.8 | 0.8 | 0.87 |
| Kpeshie           | 21.32         | 51.19              | 4.87         | 4.33       | 152.9 | 60.8 | 0.97 | 1.05 |
| Muni-Pomadze      | 4.84          | 14.94              | 2.55         | 3.65       | 139.9 | 55.8 | 0.52 | 0.9 |

Table 3. Species richness estimates at all three sites

| Sites             | Total Species Trapped (Sobs) | Singleton/Doubleton | Unique/duplicates | ACE/ICE | Chao 1/Chao 2 | Jack 1/Jack 2 |
|-------------------|-----------------------------|---------------------|-------------------|---------|---------------|---------------|
|                   |                             |                     |                   |         |               |               |
| Sakumono          | 199                         | 79/48               | 19/35             | 246/785 | 264/204       | 218/202       |
| Kpeshie           | 214                         | 94/51               | 25/56             | 268/814 | 231/219       | 239/212       |
| Muni-Pomadze      | 196                         | 81/67               | 19/51             | 192/598 | 244/199       | 215/183       |

Table 4. Educational level breakdown

| Educational Level | Sakumono | La Trade Fair | Mankoadze | Percentage (%) | Total |
|-------------------|----------|---------------|-----------|----------------|-------|
| Primary           | 15       | 8             | 23        | 16.4           | 46    |
| Middle/JHS       | 24       | 18            | 25        | 23.9           | 67    |
| Secondary/SSS     | 22       | 20            | 20        | 22.14          | 62    |
| Voc/Com/Tech      | 16       | 15            | 14        | 16.1           | 45    |
| Tertiary          | 14       | 17            | 9         | 14.3           | 40    |
| No formal education | 9       | 2             | 9         | 7.14           | 20    |
| Total             | 100      | 80            | 100       | 100            | 280   |
Educational level

Table 4 summarized the educational level of respondents. Sixty-seven individuals (23.9%) sampled had Middle or Junior High School education, 22.14% had Secondary School Education, and 16.1% had Vocational, Commercial and Technical Education.

Discussion

Insect abundance

Of the 5541 individual insects sampled, a total of 22 orders belonging to 112 families were collected and identified from distinct sites along the Sakumo, Kpeshie, and Muni-Pomadze lagoons. The most abundant order at Kpeshie was Diptera followed by Hymenoptera, Hemiptera, Coleoptera, and Lepidoptera.

Hymenoptera was the most abundant insect order followed by Diptera and Hemiptera at Sakumo, while Muni-Pomadze had Hymenoptera as the most abundant, followed by Coleoptera and Diptera. Insects constitute more than half of the world's known animal species (Wilson 1992) of which Lepidoptera is the second largest and most diverse order (Benton 1995). The highest number and far more varied order recorded at all the three sites was the Hymenoptera with Kpeshie being the most diverse.

The abundance and diversity of insects at a specific habitat depends on a wide range of factors including climatic conditions, food availability for both adults and larvae and suitable nesting sites (Allan et al. 1973; Pollard and Yates 1993). Diptera was the most abundant and diverse group at Kpeshie because the site had a varied vegetation structure and botanical composition (Struthsaker 1998; Nummelin 1996). It is very likely that the same factors accounted for the differences in species composition within the area. Insect numbers from Kpeshie were higher than those at Muni-Pomadze. This may likely have been as a result of the diversity of plants as well as the thick mangroves in the catchment area of the Kpeshie lagoon which have more food resources and habitats for the persistence of insects. Hymenopterans were the most abundant insects at both Sakumo and Muni-Pomadze. Ants, which constituted the majority of hymenopterans sampled are more abundant in grassy areas than mangroves. The predominant vegetation type at both Sakumo and Muni-Pomadze is grass with few patches of mangroves. Economic activity (e.g., fishing) is very high in these areas, subjecting the lagoons to intensive fishing activity throughout the year. These human activities disturb the breeding and other naturally instinctive behaviors of insect. Many cattle were observed grazing in and around the vast grassland areas around the lagoons. Sakomo and Muni-Pomadze are designated Ramsar sites with the convergence of thousands of birds from all over the world. These birds voraciously feed on insects and other invertebrates, greatly influencing the number and diversity of insects sampled from these sites.

Very few butterflies species were recorded at Sakumo because of its grassy nature with few flowering plants where butterflies find their nectar. Kpeshie lagoon is highly polluted (Plate 8), with its surrounding area creating ideal conditions for breeding mosquitoes, thus the very high numbers of Culcidae. The many heaps of waste provided ideal breeding sites for these mosquitoes. Many dipterans such as the Muscidae and Calliphoridae thrive on rotten matter which was also in abundance. Simulidae were found where there were ripples created by the presence of large boulders at Kpeshie. This was not the case at Sakumono and Muni-Pomadze.

Observed species richness

The fact that Kpeshie had higher species diversity is not consistent with the theoretical expectations of the species-area relationship, where smaller cities tend to support fewer species (May and Stumpf 2000). Despite the fact that Kpeshie had the highest human influence due to encroachment from a large number of both individuals and businesses, it recorded the highest indices for Simpson (1/D) and Shannon Weiner (H), Margalef (D) and Pielou (J) for both seasons. The lagoon has been partially filled with sand for development and this has drastically reduced the natural size of the lagoon, to a fraction of what it used to be. Due to the proximity of residential and commercial buildings to the lagoon, solid wastes easily finds its way into the lagoon while liquid waste is discarded directly into the lagoon from drains from all the nearby settlement as well as those around the lagoon. All these notwithstanding, the Kpeshie lagoon recorded the highest insect numbers with the most dominant species being Odontomachus spp (Formicidae), Aedes vexans (Culcidae) and Simulium venustum (Simulidae).

Relatively fewer insect species were collected at Sakumo. Although the lagoon is in the middle of a vast wetland, vegetation is very sparse, with grass covering almost the entire catchment area. The variations of temperature recorded in the dry and wet seasons were high compared to Kpeshie which has more mangroves.
Social survey

Sex of respondents
A higher percentage of the 280 respondents, 143 (51%) were women, with the remaining 137 (49%) being men.

Educational level
Approximately 24% of the respondents had middle School or Junior High School (JHS) level of education, while 22.14% of the respondents had secondary or Senior High School (SHS) level education. Fourteen percent (14%) had tertiary education while 7.14% had no formal training. Due to the new infrastructural development around the Kpeshie and Sakumo lagoons, urban dwellers are fast moving into these sites, hence, the number of respondents sampled for tertiary education was 14% for Sakumo and 17% for La Trade Fair area (Kpeshie) respectively with Mankoadze having the least with only 9%. Mankoadze observed the highest number of middle and JHS leavers with 25% respondents followed by 24% of people for Sakumo and 18% of people for La Trade Fair. Sakumo and La Trade Fair area had the highest number of respondents with vocational, commercial and technical educational status. It is obvious from these figures that formal education did not translate into prudent sanitation and waste disposal habits since about 97% of all respondents had at least some form of formal education.

Waste disposal and sanitation
Waste disposal and sanitation are significant challenges in Ghana, and this was manifested in all the study sites. For instance, the La Pleasure Beach Hotel (adjacent to the Kpeshie Lagoon) transports its wastewater to an activated sludge system located near the lagoon. Kpeshie Lagoon is the receiving water body for various drains in the Kpeshie catchment area (Figure 2). Water that enters the lagoon has its sources from communities within Burma Camp, La, Tebibiano, Teshee Camp, Africa Lake (all communities in the catchment area) and from the mangrove swamp surrounding the lagoon. Wastewater from Burma Camp is channeled through sewers into a waste stabilization pond near the Kpeshie lagoon (Kpanja 2006). This situation introduces a lot of solid matter and pollutants into the Kpeshie lagoon with its long-term impact on biodiversity including insects within the lagoon.

The social survey showed that a substantial number of inhabitants within the study area had no access to necessary sanitation facilities such as toilets and appropriate waste disposal mechanisms. As indicated in Figure 2, 71% of the respondents admitted that they had no access to private toilet facilities. About 45% of the respondents use bushes, river banks and refuse dumps as defecating grounds due to the absence of toilets facilities in their homes. Fifty percent (50%) of public toilet users expressed dissatisfaction with the unhygienic state of those facilities. During sampling, there was indiscriminate human fecal matter scattered all over most of the sampling sites especially Kpeshie and parts of Mankoadze. Only a few of the sampled communities had official refuse dump sites, and some of these official sites were located close to the wetland environments and not well maintained. For example, at Mankoadze and Trade Fair areas, one of such official dumping sites had been located extremely close to the lagoons. This observation supports the findings of a study by Noye-Nortey (1990) and Akuffo (1998), who reported that sanitation is the least managed problem in developing countries with much of the pollution coming from domestic rather than industrial sources. The neglect of good hygiene practices makes it extremely difficult in controlling water pollution in developing countries. Such situations further put undue stresses on biodiversity within these wetlands. The presence of some insect species is an indicator of pollution within water environments. In this study, the many insects sampled within the wetlands indicated various degrees of pollution as a result of the impact of anthropogenic activities, coupled with environmental conditions within these wetlands.

Conclusion
We conclude from this study that biodiversity within the three studied wetlands have been impacted negatively by human activity. This is because our sampled taxa (insect diversity within these wetlands) showed groups that prefer highly polluted environments. Our observations and results can be attributed to the dire human conditions in communities in which these wetlands are located. This is further aggravated by the lack of decent toilet facilities and managed refuse dumping sites in most of these communities forcing the inhabitants to openly defecate and dump refuse in sensitive places such as along the banks of these lagoons. This situation is further compounded by the lack of or unwillingness of local governments to manage or protect the three studied wetlands two of which are designated Ramsar sites. Bad farming practices, improper domestic and industrial waste disposal and bad fishing practices were identified as the main sources of pollution of these wetland environments. If the trend is not arrested, there are indications of further destruction of these wetlands and other such wetlands in Ghana due especially to rapid urbanisation and socioeconomic changes. Some of these threats from urbanisation especially in the Sakumo wetland environment and its catchment area come from building developers who build close to the catchment area of the lagoon.

REFERENCES
Acquah-Lamptey D, Kyerematen R, Owusu EO. 2013. Dragonflies (Odonata: Anisoptera) as tools for habitat quality assessment and monitoring in Ghana. J Agric Biodiv Res 2 (8): 178-183.
Adu-Acheampong S, Bazelet CS, Samways MJ 2016...Extent to which an agricultural mosaic supports endemic species-rich grasshopper assemblages in the Cape Floristic Region biodiversity hotspot. Agric Ecosyst Environ 227: 52-60.
Akuffo SB. 1998. Pollution Control in a Developing Economy. A Study of the Situation in Ghana. 2nd ed., Ghana Universities Press, Accra.
Allan RK. 1973. Willstatter-stoll theory of leaf reflectance evaluated ray tracing. Appl Optics 12 (10): 2448-2453.
Anon. 2001. Report of the Ad Hoc Technical Expert Group on Forest Biological Diversity (UNEPI/CBD/SBSTTA/7/6).
Barber EB, Acreeam M, Knowler D. 1997. “Economic Valuation of Wetlands: A Guide for Policymakers and Planners.” Cambridge, England: Ramsar Convention Bureau, Department of Environmental Economics and Management, University of York, NY.
Identification and characterization of Flavobacteriaceae from farmed *Oreochromis niloticus* and *Clarius gariepinus* in Uganda

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Abstract. Racheal A, Kasanga CJ, Byarugaba DK. 2018. Identification and characterization of Flavobacteriaceae from farmed Oreochromis niloticus and Clarius gariepinus. Bonorowo Wetlands 2: 42-50. Bacteria under family Flavobacteriaceae (in this study were also referred to as Flavobacteria) are important pathogens of fish, people, many other animals and plants. In this study, Flavobacteria from Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarius gariepinus*) were identified and characterized from the selected farms in Uganda. Gill and skin swabs were obtained from a total of 119 fish from 19 farms and were dissected aseptically to sample internal organs. The samples were inoculated onto Sheih media and incubated at 25°C for 48 hours. The suspected isolates were identified by colon characteristics, conventional biochemical tests and API 20 NE kits. The isolates were grouped into eight based on colon characteristic similarity. One isolate was selected per group for 16S rRNA gene sequencing and identified using the Ezbiolodnet ID software. Phylogenetic analysis of selected isolates was performed using the 16S rRNA gene sequences in BioEdit and MEGA 7.0.2 software. Basing on extrapolation of sequence analysis of the selected isolates, out of the 86 isolates, *Myroides marinus* was the most predominant species taking up 4 of the 8 groups (60 isolates) in 13 farms. The rest of the groups comprised of; *Acinetobacter pitti*, one group (6 isolates) in 6 farms, *Chryseobacterium gambrini* 2 groups (3 isolates) in 3 farms and one isolate was unidentified, in 3 farms. However, a total of 16 isolates did not grow on sub culturing. Phylogenetic analysis indicated that *M. marinus* isolates grouped with other *M. marinus* isolates from gene bank with significant intra-species diversity which was also observed with C. gambrini isolates. All the sampled farms had at least one isolate of a Flavobacterium from Tilapia and/or Catfish. Pathogenicity studies should be conducted on the isolates to establish their importance as fish pathogens and transmission dynamics so that an appropriate control measure can be recommended.

Keywords: Clarius gariepinus, Flavobacteriaceae, Oreochromis niloticus

INTRODUCTION

Agriculture is the backbone of Uganda’s economy with aquaculture as one of the major enterprises highly growing, yet still with enormous potential for production (NDP11 2015/2016-2019/20). However, increase in aquaculture is accompanied with an increased risk of diseases. Earlier it was observed during a research that over 70% of fish farms in central and western Uganda sampled with farmed tilapia and catfish had a high incidence of four bacterial pathogens including *Pseudomonas sp.*, *Aeromonas sp.*, *Vibrio sp.* and *F. columnare* of family Flavobacteriaceae (Walakira et al. 2014).

All over the world, there are numerous species of Flavobacteriaceae having the ruinous affect on the wild as well as on farmed fish stocks. Flavobacterial disease eruptions are infamously challenging to avert and control even though a lot of research has been carried out for nearly 100 years. They are known for their great economic and ecological effects (Wagner et al. 2002; Welker et al. 2005). Fish that recover from some Flavobacterial diseases remain carriers and shed the bacteria into the environment which makes them more dangerous in aquaculture (Welker et al. 2005).

Phylogenetic analysis of Flavobacterial fish pathogens is critical for the appropriate control of infections caused especially given the fact that Uganda is having a high growth rate in aquaculture (MMAIF 2004). Information about the occurrence of Flavobacterial diseases in Uganda is not well documented but there are several undocumented cases (unpublished, NAFIRL, Kajansi). The occurrence of diseases caused by Flavobacterial pathogens in countries with high aquaculture production like America, Europe and Asia (Shotts and Starliper 1999; Farmer 2004; Zamora et al. 2012a,b; Loch and Fasial 2014), could be one of the indications that Uganda will at one time face the same problem. It is therefore important to proactively study the occurrence of species prevalent in the country and with further studies on their pathogenicity. It may be possible to develop and implement appropriate control measures such as vaccination using tailored vaccines.

Specific objectives are to determine the occurrence of Flavobacteriaceae in *Oreochromis niloticus* and *Clarius gariepinus* in the selected farms in Uganda and to determine the molecular characteristics of Flavobacteriaceae isolates from *Oreochromis niloticus* and *Clarius gariepinus* in the selected farms in Uganda, using the 16S rRNA gene.
MATERIAL AND METHODS

The study area
The study was conducted on selected farms in the districts of Wakiso, Kampala, Lira, Arua, Nebbi and Kole (Kole is a new district that has just been formed from Lira district) (Figure 1).

Study design
This was a cross sectional study to isolate and identify Flavobacteriaceae isolates from African catfish and Nile tilapia in selected farms in Uganda. Bacteria were isolated from fish collected between October 2016 and March 2017. These were identified as Flavobacteria basing on growth colony characteristics (color, elevation, margin texture, colony consistency), biochemical tests and sequencing of the 16S rRNA gene.

Sampling
Convenience and purposive sampling techniques were used in this study. Purposive sampling was done based on disease history, presence of disease, availability of farms and accessibility to the farms. A total of 119 fish were collected from 19 farms. Live fish in water troughs were transported to the College of Veterinary Medicine Animal Resources and Biosecurity (COVAB) Central Diagnostic Laboratory (CDL), Makerere University, Kampala.

Isolation of bacteria under family Flavobacteriaceae
Samples of internal organs were taken aseptically including kidneys, liver, spleen and these were homogenized by cutting into smaller pieces using sterile surgical blade and then inoculated into sheih broth. Swabs were also obtained from skin, lesions and gills using a sterile swab stick and inoculated on Shieh’s agar. The samples were incubated at 25 for 48 hours. Liver, kidney and spleen were pooled into Shieh broth for 24 hours before culturing on Shieh agar supplemented with tobramycin at a concentration of 0.001g/L.

Morphological identification of Flavobacteria colonies
The phenotypic characterization of the isolates was designed on the basis of colony morphology, Gram staining and standard biochemical tests along with their consistency. All yellow bacterial colonies were considered for the study. Shieh agar and Shieh broth were made for the bacterial growth as in the table in the appendix 1. Cellular morphology was determined by Gram staining and viewed under a microscope whereby gram-negative rods were considered (magnification, x 100).

Identification of Flavobacteria by biochemical tests
Colonies were grown in peptone water for 48 hours and motility was determined under light microscope (magnification, x 100). Other biochemical tests included; presence of flexirubin type pigments using 1% KOH, cytochrome oxidase, catalase, TSI (Triple Sugar Iron Agar) tests (Sebastião et al. 2010). API 20NE test kits from Biomerieux were also used both at Makerere University and Norwegian University of Life Sciences (NMBU) as screening tests to further identify some isolates before sequencing.

Molecular identification of Flavobacteria
The isolates were preserved on Sheih agar slants and transported at room temperature to the microbiology laboratory at the Norwegian University of Life Sciences. The bacteria were sub-cultured on agar (BHI agar media was used from DIFCO Laboratories, and Merek KGaA Germany and the suspected Flavobacteriaceae colonies were divided into eight groups basing on colony morphology similarity (basing on colony color, size, elevation, margin) and one colony per group was selected for sequencing.

DNA extraction for Flavobacteria sequencing
Genomic DNA was extracted from the 8 selected isolates at the Gen-lab NMBU where further molecular analysis was performed. Genomic DNA isolation was done using QIAamp DNA mini kit (Qiagen). The manufacturer’s protocol was followed as stated in the appendix 2 and all spin steps used a bench top Minispin centrifuge.

PCR process for the extracted DNA
The 16S rRNA genes were amplified by PCR using universal bacteria primers 27f (5’AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-GGTTAACCCTTGGTACGACTT-3’). Each PCR reaction was performed in a final volume of 25µL containing: 2.5µL of 10X reaction buffer (50MM, 75MM Tris-HCL pH 9.0), 2MM MgCl2, 20MM (NH4)2SO4, 0.5 µL 10MM deoxyribonucleotide mix, 0.2 µL of DNA template, and 16.8 µL of sterile ultrapure water. PCR reactions were performed by icycler (from BIO-Rad) under the following conditions: Initial denaturation at 94 °C for 3 mins,
followed by 30 cycles of amplification as follows; denaturation at 94°C for 30s, annealing at 56°C for 30s and extension at 72°C for 2 mins, followed by a final extension step at 72°C for 5 minutes and left to stand at 4°C until analysis.

**Electrophoresis**

The PCR products were then run on 1% ultra-pure agarose (Invitrogen, Thermo Fisher Scientific) using Power Pac 300 (BioRad) at 100Volts for 60 minutes with Gene Ruler™ 1 kb Ladder. The gels prestained with syberSafe (source) were visualized using Safe Imager™ (Invitrogen) and bands of interest excised with a scalpel blade. Gel pictures were captured using ChemiDoc™ XRS Molecular imager (Bio Rad).

**Purification of the PCR products and sequencing**

The PCR products were purified using QIAquick Gel extraction kit (Quiagen) following manufacturer’s instructions as stated in appendix 2. The Purified PCR were quantified, and quality checked using Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific inc.) and sent for sequencing by sanger sequencing technology and technique at GATC Biotech, Germany using the same primers as those used for PCR.

**Data analysis**

Data was summarized and stored in Microsoft excel version 10. BLAST searches were done online to get similar sequences from the gene banks using NCBI website. The obtained sequences from isolates in this study were edited using bioedit and aligned with those retrieved from gene banks using Clustal W algorithm in MEGA version 7.0 software. The alignments were used to construct phylogenetic tree using Neighbor Joining method using Kimura-2-parameter model. Identification of the sequences was also done using EZBiocloud.net ID software online.

**RESULTS AND DISCUSSION**

**Biodata for the sampled farms**

Data of the sampled farms can be seen in Table 1.

**Symptoms encountered in the fish samples**

Both symptomatic and asymptomatic fish were sampled and some of the lesions encountered in the symptomatic fish included: hemorrhages on skin, fins, barbells, yellow skin, skin erosions, swollen belly, eroded tail fin, pale liver. Figure 2 shows some of the lesions.

**Table 1. Biodata of the selected farms**

| Status of farmer | No. of units | Species of fish | Sources of water | History of disease | Culture systems |
|------------------|--------------|-----------------|------------------|--------------------|-----------------|
| 16 small scale farms | 2-5 units for small scale | Koi carp | Lake | 5 farms (26.31%) with disease outbreak/history | 13 farms with only earthen ponds |
| | | Silver carp | River | | 3 farms with only cages |
| | | | Underground | | |
| | | | Streams | | |
| 3 large scale farms | over 20 units for large scale | African catfish | | | 2 farms with tanks and ponds |
| | | Tilapia | | | 1 farm with tanks only |

**Figure 2. Lesions encountered on catfish**
Figure 3. Colony characteristics of the study isolates. A. Isolate 1. Soft, sticky, bright yellow, flat, large size, smooth; B. Isolate 2. Soft, large, yellow, flat, gelatinous; C. Isolate 3. Yellow, medium size, flat, glistening; D. Isolate 5. Pale yellow, round, flat, medium size, shiny; E. Isolate 6. Soft, sticky, yellow, flat, medium size, irregular; F. Isolate 7. Large, yellow, round, smooth, flat, soft; G. Isolate 8. Small, orange, round, raised

Culture and isolation of flavobacteria

Culturing the pooled organs in Sheih broth followed by streaking the broth on Sheih agar always gave fewer types of colonies (sometimes only one) per sample compared to direct streaking of the gill and skin swabs on agar. A total of 86 isolates were got from the 119-fish sampled, with colonies ranging from pale yellow, bright yellow, deep yellow to orange and had a fruity odor and of varying sizes ranging from small to large. The 86 isolates were grouped into 8 groups based on colony growth characteristic similarities (color, elevation, margin texture, size of colonies) and one representative isolate from each group was considered for sequencing.

Colony characteristics

A total of 86 isolates were got with colonies ranging from pale yellow, bright yellow, deep yellow to orange and had a fruity odor and of varying sizes ranging from small to large. These were grouped into 8 and one colony per group selected. Figure 3 shows some of the colonies selected for sequencing but missing the colony for isolate 4.

Biochemical test results

Biochemical test results for the sequenced isolates

The biochemical tests results are summarized in Table 2. Some colonies produced H₂S but after storage and sub-culturing and their TSI test did not give off H₂S.

General biochemical test results for the groups

Table 3 summarizes the biochemical test results of the isolates in the groups from which the sequenced isolates were obtained. Some groups had only one isolate (i.e groups 6 and 5) while one group had two isolates (group 8). The group from which isolate 8 was got had two isolates but biochemical tests results of the other isolates are missing. Isolate 8 thus has a star in the table to indicate missing results.
**API test results**

The API test results shown in the Table 4 were for some selected isolates most of which were not sequenced directly or did not regrow on sub culturing thus. Some isolates tested using the API 20NE kits gave codes which had unacceptable profiles and therefore were not identified as shown in Table 4.

**Table 2. Biochemical test results of the sequenced isolates**

| Isolate | Catalase | Oxidase | Flexirubin Pigment | Congo red | Starch | Urease | Indole production | Motility | Gas on glucose | Glucose fermentation | Sucrose fermentation |
|---------|----------|---------|--------------------|-----------|--------|--------|------------------|----------|----------------|---------------------|----------------------|
| 1       | +        | +       | +                  | +         | +      | +      | +                | +        | +              | +                   | +                    |
| 2       | +        | +       | +                  | +         | +      | +      | +                | +        | +              | +                   | +                    |
| 3       | +        | +       | +                  | +         | +      | +      | +                | +        | +              | +                   | +                    |
| 4       | +        | +       | +                  | +         | +      | +      | +                | +        | +              | +                   | +                    |
| 5       | +        | +       | +                  | +         | +      | +      | +                | +        | +              | +                   | +                    |
| 6       | +        | +       | +                  | +         | +      | +      | +                | +        | +              | +                   | +                    |
| 7       | +        | +       | +                  | +         | +      | +      | +                | +        | +              | +                   | +                    |

**Table 4. API 20NE results**

| Isolate | Group Identification | Percentage Identification |
|---------|----------------------|---------------------------|
| A       | NR                   | Unacceptable profile     | N/A          |
| B       | NR                   | Unacceptable profile     | N/A          |
| C       | NR                   | Unacceptable profile     | N/A          |
| D       | NR                   | C. indolgenes            | 90.6         |
| E       | NR                   | Acinetobacter sp.        | 60           |
| F       | NR                   | C. indolgenes            | 99.9         |
| G       | 1                    | Myriodes sp.             | 64           |
| H       | 1                    | Weekesia sp.             | 37           |
| I       | 3                    | C. indolgenes            | 49           |

Note: NR- Not represented in the groupings since did not grow on sub-culturing N/A- Not applicable

**Comparison of conventional and API 20NE biochemical test results**

The biochemical tests compared between the conventional laboratory method and the API 20NE kits were glucose fermentation, presences of urease activity (URE), gelatin hydrolysis (GEL) by gelatinase, oxidase activity (OX) and indole production (TRP). There were minimal differences in the test results observed between the two methods (not more than two tests out of the five tests per isolate) as observed in the Table 5.

**Electrophoresis results**

Figure 4 shows the electrophoresis results with the bands of sizes of approximately 1500bp (indicated by an arrow) obtained using universal bacterial primers 27F and 1492R.

**Table 5. Comparison of API 20NE and conventional tube test results for selected isolates**

| Isolate | Test method | GLU | URE | GEL | OX | TRP |
|---------|-------------|-----|-----|-----|----|-----|
| A       | API         | -   | +   | +   | +  |     |
| B       | Conventional| -   | +   | +   | +  |     |
| C       | API         | -   | +   | +   | +  |     |
| D       | Conventional| -   | +   | +   | +  |     |
| E       | API         | -   | +   | +   | +  |     |
| F       | Conventional| -   | +   | +   | +  |     |
| G       | API         | -   | +   | +   | +  |     |
| H       | Conventional| -   | +   | +   | +  |     |
| I       | API         | -   | +   | +   | +  |     |

**Table 3. General biochemical test results of the groups**

| Representative sequenced isolate | 1 | 2 | 3 | 4 | 5 | 6 | 8* |
|----------------------------------|---|---|---|---|---|---|----|
| Flexirubin                       | 92.9 (+) | 100 (+) | 93.3 (+) | 76.2 (+) | (+) | (+) | (+) |
| Catalase                         | 100 (+) | 100 (+) | 96.7 (+) | 100 (+) | (+) | (+) | (+) |
| Oxidase                          | 85.7 (+) | 66.7 (-) | 86.7 (+) | 70.0 (+) | (+) | (+) | (+) |
| Congo red                        | 100 (+) | 100 (+) | 93.3 (+) | 70.0 (+) | (+) | (+) | (+) |
| Urease                           | 100 (+) | 100 (+) | 60.0 (+) | 76.9 (+) | (-) | (-) | (-) |
| TSI                              | 92.9 (-) | 100 (-) | 83.3 (-) | 84.6 (-) | (-) | (-) | (+) |
| H2S                              | 100 (-) | 100 (-) | 96.7 (+) | 100 (-) | (-) | (-) | (-) |
| Gliding motility                 | 92.9 (-) | 66.7 (-) | 93.3 (-) | 84.6 (-) | (+) | (-) | (-) |
| Indole production                | 71.4 (-) | 100 (-) | 70.0 (-) | 53.8 (-) | (-) | (-) | (-) |
| Gelatin hydrolysis               | 50.0 (+) | 100 (+) | 73.3 (+) | 92.3 (+) | (+) | (+) | (+) |
| Glucose fermentation             | 92.9 (-) | 100 (-) | 96.7 (-) | 92.3 (-) | (-) | (-) | (+) |
| Gas from glucose fermentation    | 100 (-) | 100 (-) | 92.3 (-) | 92.3 (-) | (-) | (-) | (+) |
| Sucrose fermentation             | 92.9 (-) | 100 (-) | 93.3 (-) | 92.3 (-) | (-) | (-) | (-) |
Identification and occurrence of the isolates

Identification of isolates using Ezbiocloud.net

The commonest species that was isolated was *M. marinus* and the closest strain to the isolates was *M. marinus* JS 08 (GQ857652) at a percentage similarity of 99.0 to 99.79% (for the different group isolates) using Ezbiocloud.net. These were isolated on 15 farms out of the 19 sampled farms. The least common species isolated were those closely similar to *M. odoratimimus*, with closest strain as *M. odoratimimus* CCUG 39352 at percentage similarity of 86.7% and *Chryseobacterium gambrini* with closest strain as *C. gambrini* DSM 18014 at a percentage similarity of 98.37 to 97.82% (for the different selected isolates) using Ezbiocloud.net.

Table 6 shows the identification of the isolates, the health status, species of fish (*Oreochromis niloticus* (O.n) or *Clarias gariepinus* (C.g)) and site of fish from which they were isolated, culture system and water source of the farms from which the isolates were obtained.

Identification of the 86 isolates

Figure 5 shows the composition of the isolates based on extrapolation of the results of the sequenced isolates.

### Table 6. Identification of isolates and their occurrence in fish

| Isolate                  | Status of fish     | Percentage similarity and Closter strain using EZBiocloud.net | Species of fish | Site on sampled fish      | Culture System | Water source       |
|--------------------------|--------------------|----------------------------------------------------------------|-----------------|---------------------------|----------------|--------------------|
| 1 Symptomatic and        |                    | *Myroides marinus* JS 08 (99.49%)                              | *C. gambrini*   | Pooled liver, spleen, gills | Tank, pond     | Rain, tap water    |
| 2 Assympt- omatic        |                    | *Myroides marinus* JS 08 (99.79%)                              | *O. niloticus*  | Gills and skin             | Pond           | Stream             |
| 3 Assympt- omatic        |                    | *Myroides marinus* JS 08 (99.0%)                               | *C. gambrini*   | Pooled kidney, liver, spleen, skin gills | Pond           | Stream             |
| 4 Assympt- omatic fish   |                    | *Myroides marinus* JS 08 (99.79%)                              | *C. gambrini*   | Pooled organs, liver, spleen, kidney | Pond, tank     | Lake               |
| 5 Symptomatic fish       |                    | *Chryseobacterium gambrini* DSM 18014 (98.37%)                 | *O. niloticus*  | Pooled organs, skin, gills | Pond           | Stream             |
| 6 Assympt- omatic        |                    | *Myroides odoratimimus* CCUG39352 (86.7%)                      | *O. niloticus*  | Pooled organs, liver, spleen kidney | Pond, Tank     | Lake               |
| 7 Symptom- atic fish     |                    | *Acinetobacter pittii* CIP 70.29 (99.36%)                      | *O. niloticus*  | Gills, skin                | Pond, cage     | Lake               |
| 8 Symptomatic and        |                    | *Chryseobacterium gambrini* DSM 18014 (98.19%)                 | *O. niloticus*  | Pooled organs, liver, spleen kidney | Tank           | Tap water, rain water |

Phylogenetic analysis

Occurrence of flavobacteria on the farms

Out of the 19 sampled farms, *Myroides marinus* was the commonest while the unidentified isolate was the least common. The isolates were distributed on the farms as summarized in Figure 6.

Key: Neighbor-Joining method was used to imply the evolutionary history (Saitou and Nei 1987), in which the sum of branch length = 0.51734957 was shown by optimal tree. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The analysis involved 16 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 989 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

The isolates 1,2,3 and 4 were grouped with the other *M. marinus* isolates obtained from the gene bank. Isolate 2 and 3 were more closely related to each other and to the reference strain *M. marinus* JS 08 compared to isolate 1 and 4. Isolate 4 was furthest from the reference strain of all the *M. marinus* isolates. Therefore, there is diversity in the phylogenetic relatedness between the isolates 1,2,3 and 4. Isolate 6 did not cluster with any of the other isolates. Isolates 8 and 5 were grouped with the other *C. gambrini* isolates obtained from the gene bank. Isolate 5 was more closely related to the reference strain compared to isolate 8.
The graphical views showing comparison of the isolates to their reference strains are shown in appendix 4. The isolates 1, 2, 3 and 4 differed from the reference strain *M. marinus* JS 08 GQ857652 at regions between 221 and 223, 591, but most especially between 1097 and 1302. Isolate 4 had the greatest differences of the four isolates. The isolates 5 and 8 differed from the reference strain *C. ganbrini* JGI1096583 in the regions between 270 and 277, 978 and 996. Isolate 8 had more nucleotide differences to the reference strain compared to isolate 5.

**Discussion**

Flavobacteria are some of the major fish pathogens of importance in aquaculture worldwide (Wakabayashi et al. 1989; Shotts and Starliper 1999; Nematollahi et al. 2003; Bernardet et al. 2006; Starliper 2011; Loch and Fasial 2015). Previous studies in Uganda by Walakira et al. (2014) indicated that *F. columnare* had a high prevalence in the selected farms in central and western Uganda. This study determined the occurrence of Flavobacteria in fish farms and their molecular characterization as a way to better understand the Flavobacterial diseases.

In this study, all the selected farms had at least one bacterium from the family *Flavobacteriaceae* isolated and some had more than one colony type of the isolates. Some of these Flavobacteria like the *Myroides* species, have potential to cause disease in laboratory experiments but have not yet been reported to cause disease in the natural (Chinnarajan et al. 2015). Sixteen isolates did not grow on sub culturing and thus were not represented in the sequencing of the selected isolates in this study.

Many genera have emerging pathogens that include *Chryseobacterium*, *Tenacibacterium*, *Ornithobacterium*, *Elizabethkingia* and these include pathogens of reptiles, humans, birds, mammals and those of fish health importance (Loch and Fasial 2015).

Seven out of the eight selected representative isolates in this study were closely related to family *Flavobacteriaceae*, grouped under the genera *Myroides* and *Chryseobacterium* as shown by the phylogenetic tree in Figure 7. These are some of the genera with the commonest species that have been reported to be associated with sick fish and even causing disease in fish (Loch and Fasial 2015).

Sixteen out of the 19 farms in this study were small scale farms some of which were getting water source from the wild. Previous studies of problems facing small scale farmers in Asia, Particularly Thailand ranked disease second to lack of funds (Chinabut et al. 2002).

Eight groups of the isolates were made during the present study on the basis of similarity in morphology of the colony and only one per group was sequenced. This was due to limited resources, but it would have been better if each isolate had been sequenced and identified individually because there is a possibility that different species or strains were grouped together. Some isolates identified as same species were morphologically different (Figure 3) and had some differences in their biochemical reactions for the tests that were carried out (Table 2), for example, isolates 1, 3, 2 and 4 that still turned out to group with the reference strain *Myroides marinus* JS 08 (bootstrap values above 60%) and were identified as *Myroides marinus* (Table 6).
The colony morphological and biochemical differences could be due to differences in the strains which was not well studied here. The fact that some of the isolates had phylogenetic relationship and yet were found in different farms in different parts of the country, could be an indication of similar source. Most of the sampled farms had previously received fingerlings from Kajansi through a government project to support fish farmers in Uganda, thus could be a common source. Isolate 6 was not closely related to any of the other isolates in this study, not even to *M. odoratimimus* which was the closest possible species. Although the closest strain was *M. Odoratimimus*, the percentage similarity of 86.7% is low and thus the isolate is a bacterium probably not under family Flavobacteriaceae.

Isolate 7 although with colony and biochemical characteristics similar to Flavobacteria, was identified as *Acinetobacter pittii* using EZtaxon ID software. The biochemical tests of many colonies in this study tentatively suggested *F. columnare* but were ruled out by the API kits and 16S rRNA gene sequencing. There were differences in the biochemical characteristics of isolates between and within the groups formed as shown in Tables 3 and 4. This could be because of differences in species or strains among the isolates in each group. The colony characteristics (color, size, elevation colony margins) similarity used to group the isolates is not sufficient to differentiate the bacteria species or strains of Flavobacteria. For example, isolates 1, 2 and 3 were all identified as *M. marinus* but have different colony growth characteristics as shown in Figure 3. Graphical views in the appendix 3 revealed differences in their nucleotides between the isolates 1, 2, 3 and 4 and thus could be due to differences in the strains.

Similarly, isolates 5 and 8 where both identified as *C. gambrini* but had differences in biochemical test results for example isolate 8 fermented glucose, produced acid on TSI and did not have gliding motility while isolate 5 did not ferment glucose, no acid production in TSI and had gliding motility.

API 20NE kits when used in this study could rule out *F. columnare* even though morphological and biochemical tests suggested otherwise. The comparison of identification by API kits and 16s RNA gene sequencing was not well studied here, although both API kits and 16S RNA gene sequencing did not identify any of the major Flavobacteria. There were minimal differences in the five test results observed between the two methods (not more than two tests out of the five tests per isolate) as observed in the Table 5. However, the number of samples tested, and the number of the biochemical tests compared were both too small to be reliable for a consequence.

Distinct findings were furnished in this study as comparable from those of the previous studies done in Uganda which have indicated a high incidence of *F. columnare* (Walakira et al. 2014). In this study, there is however a high occurrence of bacteria under family Flavobacteriaceae with the exception of *F. columnare*. There is a possibility that the presumed *F. columnare* in Walakira et al. (2014) study could have been different species under the different genera of family Flavobacteriaceae. The physiological, morphological and biochemical analysis of the suspected *F. columnare* colonies in that study probably led to a misdiagnosis. The diagnosis of lesser - known Flavobacteria in fish is difficult and laborious (considering *F. columnare*, *F. branchiophilum* and *F. psychrophillum* as the major Flavobacteria (Loch and Fasial 2015). This is because there are few diagnostic reagents specific for the lesser-known fish associated Flavobacteria organisms. Diagnosis is further made more difficult by the fact that Flavobacteria are being discovered at a high rate and their classifications keep on changing (Bernardet et al. 1996; Qu et al. 2009; Lee et al. 2010, Yoon et al. 2011; Loch and Fasial 2015). Varga et al. (2016) similarly conducted a survey for incidence of *F. columnare* in wild and cultured freshwater fish species in Hungary. Total twenty-five isolates from wild and cultured freshwater fishes were identified as *F. columnare* by using specific PCR. However, both the fragment lengths and the results of PCR-RFLP genotyping with BsuRI (HaeIII) and RsaI restriction enzymes were not convincing enough regarding *F. columnare* classification. Sequencing of the 16S ribosomal RNA gene revealed that 23 isolates belonged to the species *F. johnsoniae* and two represented *Chryseobacterium* spp. thus showing that misidentification of Flavobacteria is easily possible (Varga et al. 2016).

The commonest of the Flavobacteria isolated in the selected farms in this study was *M. marinus* as indicated in Table 3 and Figures 2 and 3. The isolates were got from both symptomatic and asymptomatic fish for example isolates (Table 6). Clinical signs in the symptomatic fish included skin erosions, hemorrhages, yellowing of the skin, swollen belly and fin erosions as shown in Figure 2. Some of the isolates from symptomatic fish with skin erosions for example isolates 1 and 8 were recovered from catfish fingerlings (*Clarias gariepinus*) that were reported to be experiencing abnormal mortalities for a week. The isolate 8 was identified as *C. gambrini*. Loch in his study stated that *Flavobacterium* sp. and *Chryseobacterium* sp. were an extensive cause of fingerling and fry mortalities in Michigan (Loch 2014). For this case however, it requires further experimental studies to tell if the isolates were the causative
agents for the skin erosions and death of the catfish fingerlings since there is a possibility of mixed infection.

A previous study by Loch has shown different Flavobacteria species being isolated from both symptomatic (with hemorrhages, skin and fin erosions, gill necrosis) and asymptomatic fish, some of which were just emerging fish pathogens (Loch 2014). Other than the three-main fish disease causing Flavobacteria, other emerging Flavobacteria have also been found to cause hemorrhages, erosions on the skin and fins (Loch and Fasil 2015). The Original Flavobacteria known to be causing fish health issues were the F. columnare, F. branchiophilum, F. psychrophilum but there are many other Flavobacteriaceae causing disease in fish. The newly identified Flavobacteria vary in the degree of virulence for example, C. aahli sp. Nov., was found to be mildly pathogenic to fish under laboratory conditions while F. sparti sp. nov., was rather more pathogenic (Loch 2014). Thus, it is important to study pathogenicity of emerging Flavobacteria.

Some farmers reported poor growth of fish. This could be due to many other factors that could include but not limited to poor management, genetic factors, reproduction in Tilapia and diseases. However, Flavobacteriosis is one of the diseases that could lead to poor growth of fish that survive the infection. Acute Flavobacteriosis was reported to contribute to poor growth in fish that survive which sometimes present with spinal abnormalities (Austin and Austin 2007).

**Conclusion**

All the sampled farms had at least one isolate of Flavobacterium from Tilapia and/or Catfish. Myrroides marinus was common in the selected farms in this study isolated on 13 farms which is 68.4% of the 19 farms. However, C. gambrini (on 4 farms) and the unidentified isolate 6 (on 3 farms) were not very common in the selected farms. None of the major Flavobacteria (F. columnare, F. branchiophilum and F. psychrophilum) was identified in this study. The routinely used biochemical and morphological growth characteristics were not sufficient to identify Flavobacteria. Phylogenetic analysis indicated that M. marinus isolates grouped with other M. marinus isolates from gene bank although intra-species diversity was observed, a similar situation observed with C. gambrini isolates.

**REFERENCE**

Adley CC, Saeb FM. 2005. Comparison of biomeriueux API 20NE and Remel RapiD NF Plus, identification systems of type strains of *Ralstonia pickettii*. Lett Appl Microbiol 41 (2): 136-140.

Austin B, Austin DA. 2007. Bacterial fish pathogens and proposal of *Flavobacterium pyrophilum* sp. nov., isolated from the gills and fin of the white mullet (*Mugil cephalus* Linnaeus, (1758)). J Microbiol 88: 22-28.

Farmer B. 2004. Improved methods for the isolation and characterization of *Flavobacterium columnare.*[Dissertation]. Louisiana State University, Agricultural and Mechanical College, Baton Rouge, LA.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evol 39: 783-791.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870-1874.

Lee SH, Kim JM, Lee JR, Park W, Jeon CO. 2010. *Flavobacterium fluvium* sp. nov., isolated from stream sediment. Int J Sys Evol Microbiol 60 (2): 353-357.

Loch PT, Faisal M. 2015. Emerging Flavobacterial infections in fish: A review. J Adv Res 6 (3): 283-300.

Loch PT. 2014. Identification of novel Flavobacteria from Michigan and assessment of their impacts on fish health. Michigan State Univ., USA.

Loch T, Faisal M. 2014. *Chryseobacterium aahli* sp. nov., isolated from lake trout (*Salvelinus namaycush*) and brown trout (*Salmo trutta*), and emended descriptions of *Chryseobacterium ginsenosidomutans* and *Chryseobacterium gregarium*. Int J Syst Evol Microbiol 64 (5): 1573-1579.

MAAIF [Ministry of Agriculture Animal Industry and Fisheries]. 2004. The National Fisheries Policy. 1st edition. Department of Fishery Resources. Kampala, Uganda.

Nematollahi A, Decostere A, Pasmans F, Haeusbrouck F. 2003. *Flavobacterium psychrophilum* infections in salmonid fish. J Fish Dis 26 (10): 563-74.

Qu DH, Yuan HL, Li HF, Deng CP. 2009. *Flavobacterium Caussense* sp. nov., isolated from sediment of eutrophic lake. Int J Syst Evol Microbiol 59 (11): 2666-2669.

Saitou N, Nei M. 1987. The Neighbor-Joining Method-a New Method for Reconstructing Phylogenetic Trees. Mol Biol Evol 4: 406-425.

Sebastião FA, Pilsarsi F, Lemos MVP. 2010. Isolation and molecular characterization of *Flavobacterium columnare* strains from fish in Brazil. J Bacteriol Res 2 (3): 22-29.

Shotts E, Starliper C. 1999. Flavobacterial diseases: columnaris disease, cold- water disease and bacterial gill disease (Fish diseases and disorders: viral, bacterial and fungal infections). CABI Publishing; New York (NY) 3: 559-576.

Starliper CE. 2011. Bacterial coldwater disease of fishes caused by *Flavobacterium psychrophilum*. J Adv Res 2 (2): 97-108.

Varga Z, Selleyi B, Paulus P, Papp M, Molnár K, Székely C. 2016. isolation and characterisation of Flavobacterium from wild and cultured freshwater fish species in Hungary. Acta Vet Hung 64 (1): 13-25.

Wagner BA, Wise DJ, Khoo LH, Terhune JS. 2002. The epidemiology of bacterial diseases in food-size channel catfish. J Aquat Anim Health 14: 263-272.

Wakabashy H, Huh G, Kimura N. 1989. *Flavobacterium branchiophilica* sp. nov., a causative agent of bacterial gill disease of freshwater fish. Int J Sys Bacteriol 39 (3): 213-216.

Walakira J, Akoll P, Engole M, Sterwutta M, Nkambo M, Namulawa V, Kityo G, Musimbi F, Abaho I, Kasigwa H, Mbazai D, Kahwa D, Naigaga I, Birungi D, Rutaisire J, Majalija S. 2014. Common fish diseases and parasites affecting wild and farmed Tilapia and catfish in Central and Western Uganda. Uganda J Agric Sci 17 (3): 138-157.

Walser LT, Shoemaker AC, Arias RC, Klesius HP. 2005. Transmission and detection of *Flavobacterium columnare* in channel catfish *Lctalurus punctatus*. Dis Aquat Org 63: 129-138.

Yoon JH, Park S, Kang SJ, Myung SC, Kim W. 2011. *Flavobacterium poritii* sp. nov., isolated from seawater. Int J Sys Evol Microbiol 61 (6): 1758-1767.

Zamora L, Fernández-Garayzábal J, Palacios M, Sánchez-Porro C, Svensson-Stadler L, Domínguez L. 2012. *Chryseobacterium oncorhynchi* sp. nov., isolated from rainbow trout (*Oncorhynchus mykiss*). Syst Appl Microbiol 35 (1): 24-29.

Zamora L, Vela AL, Palacios MA, Sánchez-Porro C, Svensson-Stadler LA, Domínguez L. 2012. *Chryseobacterium viscerum* sp. nov., isolated from diseased fish. Int J Syst Evol Microbiol 62 (12): 2934-2940.
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Chapter in book:

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Abstract:

Assema AD. 2007. Seed production and dispersal of Rhiza strick. 50th Annual Symposium of the International Association for Vegetation Science, Swanage, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Saturo (eds.) Toward Mount Lawu National Park. Proceedings of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000.

Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic Escherichia coli predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com. DOI: 10.1038/msb.2008.24
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