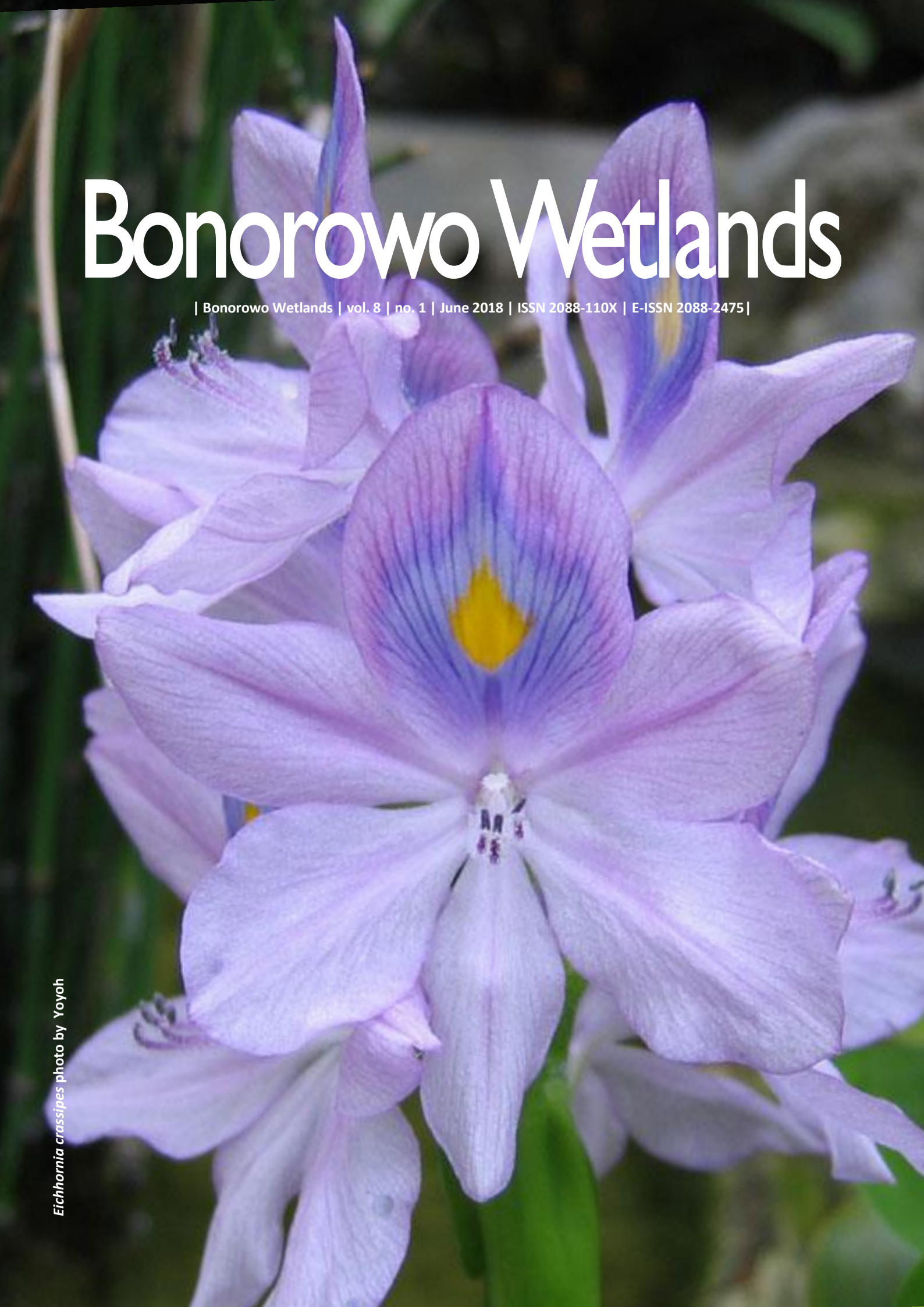

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Eichhornia crassipes photo by Yoyoh

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Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

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Proceeding:

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Thesis, Dissertation:

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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 8: 1-12.* Macrophytes are an indispensable component of any wetlands. They are the base of the trophic structure and variously affect the function of the aquatic ecosystem. To identify the causative plant species, extensive invasion of macrophytes was enforced for present studies in Ansupa Lake, the largest freshwater lake of the state Odisha (India). Regular field inspection, quadratic sampling, and specimen collections were carried out 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, species distribution, etc. 244 macrophyte species were identified, including 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 the 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 the existence of a diverse group of macrophytes. Still, the extensive invasion of a few species has threatened the lake, which needs to be appropriately managed to restore the health of this natural resource for the benefit of humanity.

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’’ restoring water quality (Byers et al. 2006). It includes flowering and non-flowering plants that start their lives in and around water bodies (Chambers et al., 2008). Some 2,614 aquatic vascular macrophytes occur globally, representing only 1% of the total number of vascular plants (Ansari et al., 2017). The total number of aquatic plant species in Indian freshwaters exceeds 1,200 (Gopal 1995). Many aquatic plant species are invasive species (Oyedeji and Abowei 2012). These plants cause local loss 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 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 site, is the largest freshwater 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-sized fishes, 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 residents live 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 July and August each year. It hosts 44 species of phytoplankton, 32 species of zooplankton, and 30 species of fishes (Patra and Patra 2007). For the first time, Panda et al. (2016) reported the 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 are few published works 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. These problematic weeds need to be appropriately managed to conserve indigenous biota and create better livelihood opportunities from the lake.

MATERIALS AND METHODS

Study area

Ansupa Lake is the largest freshwater 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 riverbank of Mahanadi (Figure 1). The lake area 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 out during November 2014 and extensive regular fieldwork from April to November 2017. The recorded macrophytes were identified with the help of available regional and international scientific literature (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., size1m × 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 ($1\text{-}D_S$) from Simpson Dominance index (Simpson 1949), Shannon-Wiener index (Shannon and Wiener 1963) and Species evenness index (J) (Pielou 1975) were calculated as follows:

$$\text{Frequecy} = \frac{\text{No. of plots in which a species occurs}}{\text{Total no of plots sampled}} \times 100$$

$$\text{Abundance} = \frac{\text{Total number of individuals of a species in all quadrates}}{\text{Number of quadrates in which the species occured}}$$

$$\text{Species dispersion or Whitford's index } (A/F) = \frac{\text{Abundance}}{\text{Frequency}}$$

$$\text{Species richness Index } (RI) = \frac{S-1}{\ln N} \quad \textit{as per Margalef (1958)}$$

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.

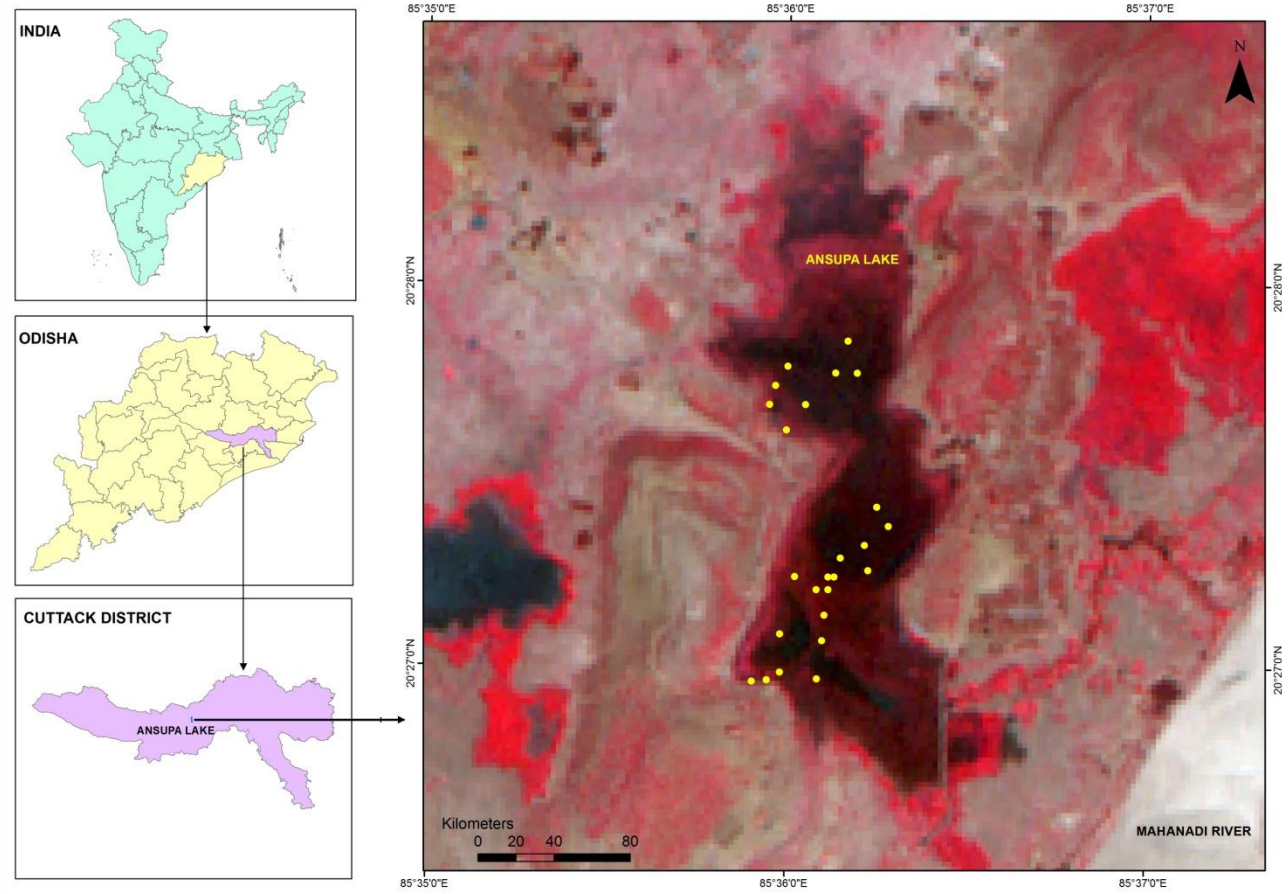

Figure 1. Location map of Ansupa Lake, Cuttack District, Odisha, India

$$Simpson\ dominance\ index\ (D_s) = \Sigma Pi^2\ \ as\ per\ Simpson\ (1949)$$

$$\text{Shannon} - \text{Wiener index} = -\Sigma\ \text{Pi}\ \log_n \text{Pi}\quad as\ per\ Shannon - Weiner\ (1963)$$

$$\text{Where, } Pi = \frac{Number\ of\ individual\ of\ one\ species}{Total\ number\ of\ all\ individuals}$$

$$Species\ Evenness\ Index\ (EI) = \frac{H'}{\ln S}\ \ as\ per\ Pielou\ (1975)$$

Where, H′ is the Shannon-Weiner index of the community and S is the total number of species in the community.

RESULTS AND DISCUSSION

A total of 244 vascular macrophytes were identified to occur in 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) except Azolla microphylla Kaulf, an annual species others were perennial in their growth form (Table 2). The angiospermic macrophytes belong to a total of sixty families. Poaceae and Cyperaceae were recorded as the most diversified families among these families (Figure 2). The classification of all the recorded macrophytes based on habitat preference showed 182 (75%) semi-aquatic species and 62 (25%) aquatic species (Figure 3). Categorization 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 the 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 of nativity resulted in 56 species out of 244 species as exotic or nonnative macrophytes of India (Table 1 and Table 2). The quadratic study revealed the quantitative status of 28 common macrophytes (Table 3). Maximum species diversity was recorded in the peripheral or shoreline plots. The most frequent and abundant species were *Ceratophylum demersum* L., *Hydrila verticelastar* (L.) Pers., *Nelumbo nucefera* Gaertn., *Najas* sp., *Utricularia* sp., *Eichhornia crassipes* (Mart.) Solm-Laub. and *Salvinia molesta* D. S. Mitch from the lake's interior. Other species like *Polygonum barbatum* L., *Hymenachne amplexicaulis* (Rudge) Nees, *Cyperus iria* L., *Alternanthera philoxeroides* A. St-Hil., *Cyperus rotundus* L. were more abundant at the land water interface (i.e., marshy areas). The distribution pattern (i.e., Whitford's index) showed all species with mthe ore or less contagious types of distribution (A/F > 0.05). The diversity indices study showed Simpson complement index-0.561, Shannon-Weiner index-1.367, Species richness index 3.079, and Species evenness index-0.156 (Figure 9).

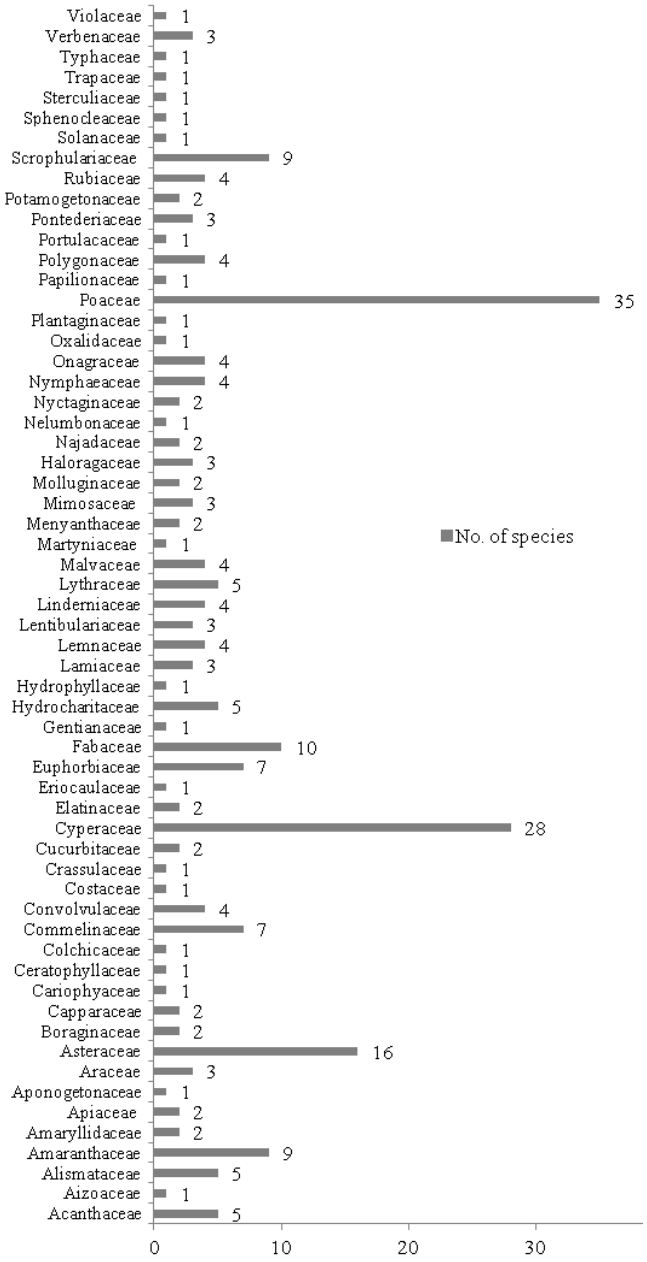

Figure 2. Family wise recorded number of angiospermic macrophytes species

The study found the occurrence of wide habitat variability that helped the establishment of a 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 the diversity index of the ecosystem, as calculated by the 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 rarely found elsewhere in the state. *Hygroryza aristata* (Retz.) Nees. Ex Wt. & Arn. and *Oryza rufipogon* Griff., the wild

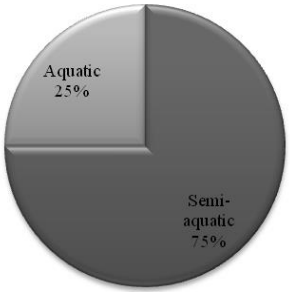

Figure 3. Classification as per habitat requirement: Aquatic and semi-aquatic plants (%)

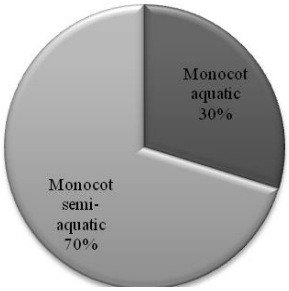

Figure 6. Classification of monocots into habitat groups: Aquatic and semi-aquatic monocots (%)

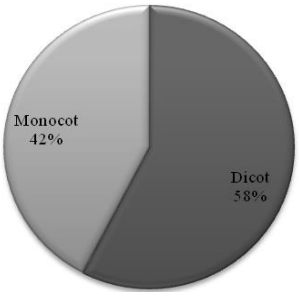

Figure 4. Classification into Angiosperm group: Diversity of dicot and monocot species (%)

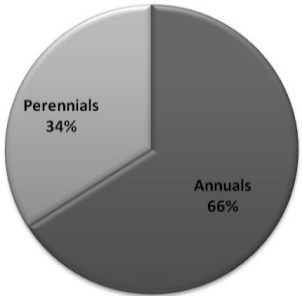

Figure 7. Classification of macrophytes into growth forms: Growth form of macrophytes (%)

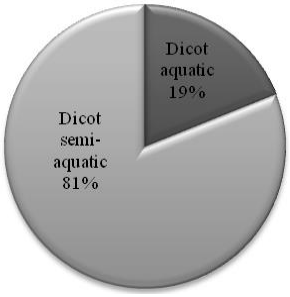

Figure 5. Classification of dicots into habitat group: Aquatic and semi-aquatic dicots (%)

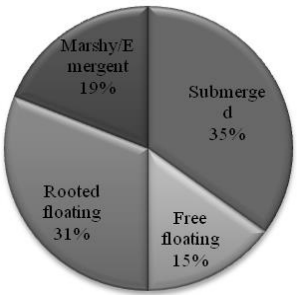

Figure 8. Classification of aquatic plants into their adaptation group: Adaptation forms of aquatic plants (%)

relative of edible rice was a common occurrence in the lake (Plate 1). The aesthetically essential 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 surrounding hills and siltation decreased water flow due to the closing of inlets and outlets with Mahanadi River; intensive fertilizer load is the possible factors for degradation of the lake.

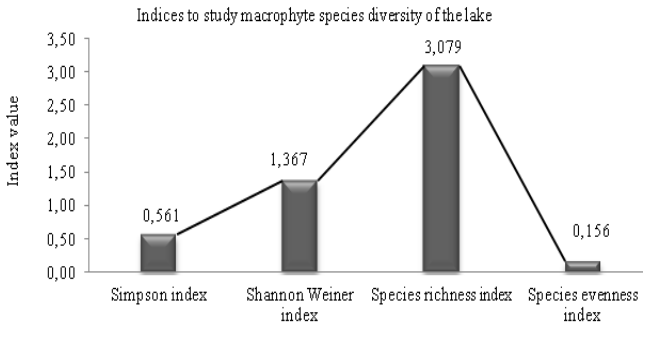

Figure 9. Diversity indices from quadrate data

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	**Trianthema portulacastrum* L.	D	Semi-aquatic	Annual
Alismataceae	7	**Alisma plantago-aquatica* L.	M	Aquatic (S)	Annual
	8	*Limnophyton obtusifolium* (L.) Miq.	M	Aquatic (S)	Annual
	9	*Sagitaria sagittifolia* L.	M	Aquatic (S)	Annual
	10	*Sagittaria guayanensis* var. *lappula* D. Don	M	Aquatic (S)	Annual
	11	*Sagitaria trifolia* L.	M	Aquatic (S)	Annual
Amaranthaceae	12	**Achyranthes aspera* L.	D	Semi-aquatic	Annual
	13	*Aerva lanata* (L.) Juss. ex Schult.	D	Semi-aquatic	Annual
	14	**Alternanthera paronychioides* 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. & Schltdl.	D	Semi-aquatic	Perennial
Aponogetonaceae	25	*Aponogeton natans* (L.) Engl. & Krause	M	Aquatic (S)	Annual
	26	*Alocasia indica* (Roxb.) Schott	M	Semi-aquatic	Perennial
Araceae	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	*Caesulia axillaris* 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	**Gnaphalium 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	**Synedrella 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	*Cyanotis axillaris* (L.) D.Don ex Sweet	M	Semi-aquatic	Perennial
	56	**Evolvulus nummularius* (L.) 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. *Fistulosa* (Mart. ex Choisy) Austin	D	Semi-aquatic	Perennial

	61	**Ipomoea pes-tigridis* L.	D	Semi-aquatic	Perennial
	62	*Merremia tridentata* (L.) Hall. f.	D	Semi-aquatic	Perennial
Costaceae	63	*Costus speciosus* (J.Koenig) Sm.	M	Semi-aquatic	Perennial
Crassulaceae	64	*Bryophyllum calycinum* Salisb.	D	Semi-aquatic	Perennial
Cucurbitaceae	65	*Mukia maderaspatana* (L.) M. Roem.	D	Semi-aquatic	Annual
	66	*Cucumis melo* L.	D	Semi-aquatic	Annual
Cyperaceae	67	*Cyperus alopecuroides* Rottb.	M	Semi-aquatic	Annual
	68	**Cyperus brevifolius* (Rottb.) Hassk.	M	Semi-aquatic	Perennial
	69	*Cyperus cephalotes* Vahl	M	Semi-aquatic	Perennial
	70	*Cyperus compressus* L.	M	Semi-aquatic	Annual
	71	*Cyperus corymbosus* Rottb.	M	Semi-aquatic	Perennial
	72	*Cyperus difformis* 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* Rottb.	M	Semi-aquatic	Perennial
	78	*Cyperus rotundus* L.	M	Semi-aquatic	Perennial
	79	**Cyperus strigosus* L.	M	Semi-aquatic	Perennial
	80	*Eleocharis acutangula* (Roxb.) schutt.	M	Aquatic (RE)	Perennial
	81	*Echinochloa crus-galli* (L.) P. Beauv.	M	Semi-aquatic	Annual
	82	*Eleocharis dulcis* (Burm.f.) Trin. ex Henschel	M	Semi-aquatic	Perennial
	83	*Fimbristylis dipsacea* (Rottb.) 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 miliacea* (L.) Vahl	M	Semi-aquatic	Annual
	87	*Fuirena ciliaris* (L.) Roxb.	M	Semi-aquatic	Annual
	88	**Kyllinga tenuifolia* Steud.	M	Semi-aquatic	Annual
	89	*Lipocarpha 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 articulatus* (L.) Palla	M	Semi-aquatic	Annual
	93	*Schoenoplectus grossus* (L.f.) Palla	M	Semi-aquatic	Perennial
	94	*Schoenoplectiella supina* (L.) Lye	M	Semi-aquatic	Annual
Elatinaceae	95	**Bergia ammannioides* Roxb. ex Roth	D	Semi-aquatic	Annual
	96	*Bergia capensis* L.	D	Semi-aquatic	Perennial
Eriocaulaceae	97	*Eriocaulon quinquangulare* L.	M	Semi-aquatic	Perennial
Euphorbiaceae	98	*Acalypha indica* L.	D	Semi-aquatic	Annual
	99	**Croton bonplandianus* (Baill.) Kuntze	D	Semi-aquatic	Annual
	100	*Euphorbia hirta* L.	D	Semi-aquatic	Annual
	101	**Euphorbia prostrata* Aiton.	D	Semi-aquatic	Annual
	102	*Jatropha gossypiifolia* L.	D	Semi-aquatic	Perennial
	103	**Phyllanthus tenellus* Roxb.	D	Semi-aquatic	Perennial
	104	**Ricinus communis* L.	D	Semi-aquatic	Perennial
Fabaceae	105	*Aeschynomene aspera* L.	D	Semi-aquatic	Annual
	106	*Aeschynomene 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	*Sesbania bispinosa* (Jacq.) W.F. Wt.	D	Semi-aquatic	Annual
Gentianaceae	115	*Hoppea dichotoma* Willd.	D	Semi-aquatic	Annual
Hydrocharitaceae	116	*Blyxa echinosperma* (Clarke) Hook.f.	M	Aquatic (S)	Annual
	117	*Hydrilla 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. Hara	M	Aquatic (S)	Annual
Hydrophyllaceae	121	*Hydrolea zeylanica* (L.) Vahl.	D	Aquatic (RE)	Annual
Lamiaceae	122	*Anisomeles indica* (L.) O. Kuntze.	D	Semi-aquatic	Perennial
	123	*Leucas aspera* (Willd.) Link	D	Semi-aquatic	Annual
	124	*Pogostemon quadrifolius* (Benth.) F. Muell.	D	Semi-aquatic	Annual
Lemnaceae	125	**Spirodela polyrrhiza* (L.) Schleid.	M	Aquatic (FF)	Perennial
	126	*Lemna gibba* L.	M	Aquatic (FF)	Annual

	127	*Lemna aequinoctialis* Welw	M	Aquatic (FF)	Annual
	128	*Wolffia globosa* (Roxb.) Hartog & Vander Plas	M	Aquatic (FF)	Annual
Lentibulariaceae	129	*Utricularia aurea* Lour.	D	Aquatic (S)	Annual
	130	*Utricularia inflexa* Forssk.	D	Aquatic (S)	Annual
	131	*Utricularia bifida* L.	D	Aquatic (S)	Annual
Linderniaceae	132	*Lindernia crustacea* (L.) F.Muell.	D	Semi-aquatic	Annual
Lythraceae	133	*Ammannia baccifera* L.	D	Semi-aquatic	Annual
	134	*Ammannia multiflora* Roxb.	D	Semi-aquatic	Annual
	135	*Ammannia 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
Malvaceae	138	*Abutilon indicum* (L.) Sweet	D	Semi-aquatic	Annual
	139	*Corchorus aestuans* L.	D	Semi-aquatic	Annual
	140	*Sida cordifolia* L.	D	Semi-aquatic	Annual
	141	*Urena lobata* L.	D	Semi-aquatic	Annual
Martyniaceae	142	**Martynia annua* L.	D	Semi-aquatic	Annual
Menyanthaceae	143	*Nymphoides hydrophylla* (Lour.) Kuntze	D	Aquatic (RF)	Annual
	144	*Nymphoides indica* (L.) Kuntze	D	Aquatic (RF)	Annual
Mimosaceae	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
Molluginaceae	148	*Glinus oppositifolius* (L.) Aug. DC	D	Semi-aquatic	Annual
	149	*Mollugo pentaphylla* L.	D	Semi-aquatic	Annual
Haloragaceae	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
Najadaceae	153	*Najas faveolata* A. Br. ex Magam.	M	Aquatic (S)	Perennial
	154	*Najas indica* (Willd) Cham.	M	Aquatic (S)	Perennial
	155	*Najas marina* L.	M	Aquatic (S)	Perennial
Nelumbonaceae	156	*Nelumbo nucifera* Gaertn.	D	Aquatic (RF)	Perennial
Nyctaginaceae	157	*Boerhavia diffusa* L.	D	Semi-aquatic	Annual
	158	*Boerhavia repens* L.	D	Semi-aquatic	Annual
Nymphaeaceae	159	*Euryale ferox* Salisb.	D	Aquatic (RF)	Perennial
	160	*Nymphaea nouchali* Burm.f.	D	Aquatic (RF)	Perennial
	161	*Nymphaea pubescens* Willd.	D	Aquatic (RF)	Perennial
	162	*Nymphaea rubra* Roxb. ex Andrews	D	Aquatic (RF)	Perennial
Onagraceae	163	*Ludwigia prostrata* Roxb.	D	Semi-aquatic	Annual
	164	*Ludwigia adscendens* (L.) H. Hara	D	Aquatic (RF)	Perennial
	165	*Ludwigia octovalvis* (Jacq.) P.H. Raven	D	Semi-aquatic	Annual
	166	*Ludwigia perennis* L.	D	Semi-aquatic	Annual
Oxalidaceae	167	*Oxalis corniculata* L.	D	Semi-aquatic	Annual
Plantaginaceae	168	**Scoparia dulcis* L.	D	Semi-aquatic	Annual
Poaceae	169	*Apluda mutica* L.	M	Semi-aquatic	Annual
	170	*Arundinella pumila* (Hochst. ex A.Rich) Steud	M	Semi-aquatic	Annual
	171	*Axonopus compressus* (Sw.) P.Beauv.	M	Semi-aquatic	Perennial
	172	*Brachiaria deflexa* (Schumach.) C.E.Hubb. ex Robyns	M	Semi-aquatic	Annual
	173	*Brachiaria mutica* (Forssk.) Stapf.	M	Semi-aquatic	Perennial
	174	*Brachiaria ramosa* (L.) Stapf	M	Semi-aquatic	Annual
	175	*Brachiaria reptans* (L.) C.A.Gardner & C.E.Hubb	M	Semi-aquatic	Annual
	176	**Chloris barbata* Sw.	M	Semi-aquatic	Annual
	177	*Cyrtococcum longipes* (Hook.f.) A.Camus	M	Semi-aquatic	Perennial
	178	*Cynodon dactylon* (L.) Pers.	M	Semi-aquatic	Perennial
	179	**Dactyloctenium aegyptium* (L.) Willd.	M	Semi-aquatic	Annual
	180	*Dichanthelium sp.*	M	Semi-aquatic	Annual
	181	*Echinochloa colona* (L.) Link	M	Semi-aquatic	Annual
	182	*Echinochloa crus-galli* (L.) P.Beauv.	M	Semi-aquatic	Annual
	183	*Echinochloa stagnina* (Retz.) Beauv.	M	Semi-aquatic	Annual
	184	*Eleusine indica* (L.) Gaertn	M	Semi-aquatic	Annual
	185	*Elytrophorus spicatus* (Willd.) A. Camus	M	Semi-aquatic	Annual
	186	*Eragrostis ciliaris* (L.) R.Br.	M	Semi-aquatic	Annual
	187	*Eragrostis gangetica* (Roxb.) Steudel	M	Semi-aquatic	Annual
	188	*Eragrostis japonica* (Thunb.) Trin.	M	Semi-aquatic	Perennial
	189	*Eragrostis pilosa* (L.) P.Beauv.	M	Semi-aquatic	Annual
	190	*Eragrostis tenella* (L.) P.Beauv.ex Roem.& Schult.	M	Semi-aquatic	Annual
	191	*Hygroryza aristata (*Retz.) Nees ex Wight & Arn	M	Aquatic (RF)	Perennial
	192	**Hymenachne amplexicaulis* (Rudge) Nees	M	Aquatic (RF)	Perennial
	193	*Leersia hexandra* Sw.	M	Semi-aquatic	Perennial

	194	*Oryza rufipogon* Griff.	M	Semi-aquatic	Perennial
	195	*Panicum sumatrense* Roth	M	Semi-aquatic	Perennial
	196	**Paspalum dilatatum* Poir	M	Semi-aquatic	Annual
	197	*Paspalum distichum* L.	M	Semi-aquatic	Perennial
	198	*Paspalum vaginatum* Sw.	M	Semi-aquatic	Annual
	199	*Setaria pumila* (Poir.) Roem. & Schult.	M	Semi-aquatic	Annual
	200	*Saccharum spontaneum* L	M	Semi-aquatic	Perennial
	201	*Setaria glauca* (L.) Beauv.	M	Semi-aquatic	Annual
	202	*Sporobolus coromandelianus* (Retzi.) Kunth	M	Semi-aquatic	Annual
Papilionaceae	203	*Sesbania bispinosa* (Jacq.) W.Wight.	D	Semi-aquatic	Annual
Polygonaceae	204	**Persicaria glabrum* (Willd.) M.Gomez	D	Semi-aquatic	Perennial
	205	**Polygonum barbatum* L.	D	Semi-aquatic	Perennial
	206	*Polygonum plebeium* R. Br.	D	Semi-aquatic	Annual
	207	**Rumex maritimus* L.	D	Semi-aquatic	Annual
Pontederiaceae	208	*Eichhornia crassipes* (Mart.) Solm-Laub.	M	Aquatic (RF)	Perennial
	209	*Monochoria hastata* (L.) Solm.	M	Aquatic (RF)	Perennial
	210	*Monochoria vaginalis* (Burm f.) Presl.	M	Aquatic (RE)	Perennial
Portulacaceae	211	*Portulaca oleracea* L.	D	Semi-aquatic	Annual
Potamogetonaceae	212	**Potamogeton nodosus* Poir.	M	Aquatic (S)	Annual
	213	*Stuckenia pectinata* (L.) Börner	M	Aquatic (S)	Perennial
Rubiaceae	214	*Dentella repens* (L.) Forst. et Forst.	D	Semi-aquatic	Annual
	215	*Oldenlandia diffusa* (Willd.) Roxb.	D	Semi-aquatic	Annual
	216	*Mitracarpus hirtus* (L.) DC.	D	Semi-aquatic	Annual
	217	*Oldenlandia corymbosa* L.	D	Semi-aquatic	Annual
Scrophulariaceae	218	*Bacopa monnieri* (L.) Pennell.	D	Semi-aquatic	Annual
	219	*Dopatrium junceum* (Roxb.) Buch-Ham. ex Benth.	D	Aquatic (RE)	Annual
	220	*Limnophila aquatica* (Roxb.) Alston	D	Aquatic (RE)	Annual
	221	*Limnophila heterophylla* (Roxb.) Benth.	D	Aquatic (RE)	Annual
	222	*Limnophila indica* (L.) Druce	D	Aquatic (RE)	Annual
	223	*Limnophila sessiliflora* (Vahl) Blume	D	Aquatic (RE)	Annual
	224	*Lindernia anagallis* (Burm.f.) Pennel	D	Semi-aquatic	Annual
	225	*Lindernia antipoda* (L.) Alston	D	Semi-aquatic	Annual
	226	*Lindernia parviflora* (Roxb.) Haines	D	Semi-aquatic	Annual
	227	*Mecardonia procumbens* (Mills.) Small	D	Semi-aquatic	Annual
	228	*Scoparia dulcis* L.	D	Semi-aquatic	Annual
	229	**Verbascum chinense* (L.) Santapau	D	Semi-aquatic	Annual
Solanaceae	230	*Physalis minima* L.	D	Semi-aquatic	Annual
Sphenocleaceae	231	*Sphenoclea zeylanica* Gaertn.	D	Semi-aquatic	Annual
Sterculiaceae	232	*Melochia corchorifolia* L.	D	Semi-aquatic	Annual
Trapaceae	233	*Trapa natans* L. var. *bispinosa* (Roxb.) Makino	D	Aquatic (RF)	Perennial
Typhaceae	234	**Typha angustata* Bory & Chaub.	M	Aquatic (RE)	Perennial
Verbenaceae	235	**Lantana camara* L.	D	Semi-aquatic	Perennial
	236	**Lippia javanica* (Burm.f.) Spreng.	D	Semi-aquatic	Perennial
	237	*Phyla nodiflora* (L.) Greene	D	Semi-aquatic	Annual
Violaceae	238	*Hybanthus enneaspermus* (L.) F.Muell.	D	Semi-aquatic	Annual

Note: D= Dicot, M= Monocot, S= Submerged, FF= Free floating, RF= Rooted floating, RE= Rooted erect, *=Exotic or nonnative species (Un-marked species are native or indigenous to India)

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
Salviniaceae	3	**Azolla microphylla* Kaulf.	Aquatic (FF)	Annual
	4	*Azolla pinnata* R.Br.	Aquatic (FF)	Perennial
	5	**Salvinia minima* Baker	Aquatic (FF)	Perennial
	6	**Salvinia molesta* D.S. Mitch	Aquatic (FF)	Perennial

Note: RF=Rooted floating, FF=Free floating, *= Exotic or nonnative 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. Hara	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.f.) 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
Nymphaea 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 Gaertn.	57	16	64	3.56	0.056
Pistia stratiotes L.	11	3	12	3.67	0.306
Spirodela polyrrhiza (L.) Schleid.	54	4	16	13.5	0.844
Utricularia sp.	171	4	16	42.75	2.672
Lemna 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
*Hygroryza aristata (*Retz.) Nees ex Wight & Arn	7	2	8	3.5	0.438
Lindernia parviflora (Roxb.) Haines	10	2	8	5.0	0.625

A **B** **C** **D**

Plate 1. Some taxonomically important taxa from Ansupa Lake, Odisha, India. Note: A. *Oryza rufipogon*, B. *Hygroryza 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 having these troublesome weeds, the lake also hosts many macrophytes used as food, fodder, or medicine by the local households. Control of invasion and their management is tedious and needs multiple strategies. Management of this invasive grass must include a combination of techniques 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 currently 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 an indispensable constituent of any wetland. They provide habitat to various aquatic fauna, act as primary producers, oxygenate the 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 the inflow of silt, reduce the nutrient load by self-utilizing, and minimize the development of algal blooms (Naskar 1990; Bornette and Puijalon 2009; Ansari et al. 2017). But, sometimes environments enforce and help invade exotic weeds in aquatic ecosystems, which negatively affect the entire ecosystem. These plants compete with native species and often facilitate the loss or extinction of less aggressive and indigenous species (Stallings et al., 2015). In many instances, they negatively affect 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 to have attained the status of aquatic weeds (Naskar 1990, Gupta 2012), 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 change in floristic composition, maintaining required depth, reducing fertilizer use in agriculture in nearby cultivation lands, and creating green coverage in surrounding barren lands can save native biota from alien species to invade many aquatic ecosystems.

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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. 2018.* Assessing the impacts of climate variability and climate change on biodiversity in Lake Nakuru, Kenya. *Bonorowo Wetlands 8: 13-24.* This study evaluates the effects 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 reviewed and analyzed noticed climatic records from 2000 to 2014. Several methods were used to ascertain the past and current climatic parameters (temperature, rainfall, and evaporation) and the physicochemical characteristics of Lake Nakuru (conductivity, phytoplankton, lesser flamingos, and the lake depth). These included time series analysis and trend analysis, so Pearson's correlation analysis was used to show a relationship between the alterations in lake conductivity to alterations in population estimates of the lesser flamingos and the phytoplankton. Data set extracted from the Coupled Model Inter-comparison Project Phase 5 (CMIP5) (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 analyzed. The results were used to evaluate the impact of climate change on the lesser flamingos 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 increment 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 increment of 22.9 km² (71.92% surface area increment). The mean conductivity of the lake also lessened, leading to the loss of phytoplankton on which flamingos feed, making them migrate. A strong positive correlation between conductivity and the lesser flamingo population was noticed, signifying that low conductivity affects the growth of phytoplankton. Since the lesser flamingos depend on the phytoplankton for their feed, this subsequently revealed that the phytoplankton density could be a notable predictor of the lesser flamingo occurrence in Lake Nakuru. A strong positive correlation was noticed between phytoplankton and the lesser flamingo population confirming that feed availability is a key determining factor of the lesser flamingo distribution in the lake. It is projected that there would be an increment in temperatures, rainfall, and evaporation for 2017 to 2100 under RCP2.6 and RCP8.5 relative to the baseline period 1971 to 2000 obtained from the Coupled Model Inter-comparison Project phase 5 (CMIP5) 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 flamingos 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 some areas in Africa evidently are highly vulnerable to climate variability and change. Kenya's current climate predictions have forecasted increased changes and variability of different climatic factors. Severe challenges to sustainable development are being propounded by climate change in Kenya, as it's possibly a significant environmental challenge of our time (Mutai et al., 2010). Focusing on the 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 crops. Various rainfall patterns have posed threats to the renowned wildlife safaris in Kenya, especially to one of the Seven Wonders of the World: The Mara River migration of wildebeests, which is familiar to tourists around the world (Climate Action Network 2009). Irregular rain patterns affect the wildebeests as the smell of rain influences their migration. The migration pattern is usually timed to show a relationship between the growth of grass and annual rainfall patterns in the North. Drawing closer to March, characterized by a season of temporary dryness, the wildebeests begin migrating from Serengeti as the grass starts drying out towards the western Serengeti woodlands. By the end of June, when the long rains commence

declining in Kenya, the arrival of wildebeest from the Western Serengeti is noticed in the Maasai Mara Game Reserve. Due to unpredictable climate, scarce feeding vegetation, and the drying-up of rivers have caused considerable losses in wildlife numbers (Climate Action Network 2009).

There is a wide 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 being endemic (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 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 this biodiversity will be critical to securing livelihoods resulting in reduced poverty levels - reflecting a population of 46.6 percent - suggesting a nine percent alteration if the social equity scales are to be attained as projected by 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). The US $1-2 billion per year was the amount this figure was forecasted to raise 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. A range of policy instruments needs to be formulated to tackle climate change. A national policy on climate change needs 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 be economically affected by the impacts of climate change and 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 to understand the causes of increased lake levels. (ii) to show a relationship between lake conductivity alterations to alterations in aquatic species' population estimates, 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 the 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 one of Kenya's important tourist destinations. Lake Nakuru National Park, Kenya is located between 0°19'- 0°24' S and 36°04'-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 scarps leading to the Mau Escarpment to the west.

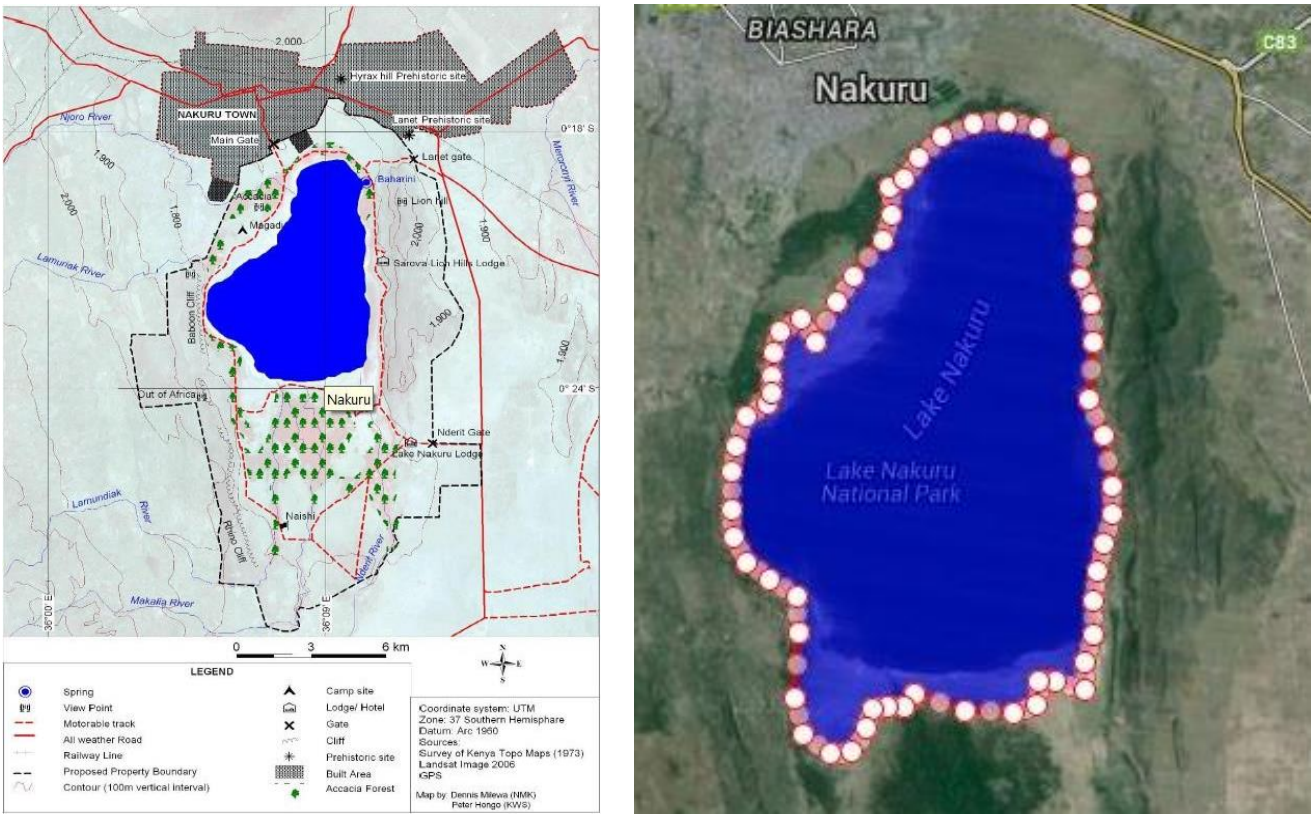
Lake Nakuru extends in the N-S direction in the trend of the axial rift faults, as shown by Figure 1. It includes other chains of alkaline-saline lakes in the eastern arm of the Rift Valley, Kenya. Existing more than twelve million years, one of the earth's spectacular geological formations was formed by the catchment and its landforms, including 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 the chemistry of the lakes' water. The lake's ecosystem is made unique by the chemistry of the alkaline water, which depends on the larger catchment for sustenance and is independent of its immediate environment for its functions. White salt filets swirling with dust devils are sometimes created when enormous water body reductions result from the lake's surface area alterations.

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 Inter-comparison Project Phase 5 (CMIP5) Representative Concentration Pathways (RCP2.6 and RCP8.5) near-surface temperature, rainfall, and evaporation data. Lake data comprised conductivity, lake levels, surface area, and depth. Flamingo data consists of the lesser flamingo population. Below is a detailed description of the data types and their sources.

Procedures

In this section, the methods used in the study for data collection, organization, and analysis based on the study's specific objectives are propounded.



A **B**

Figure 1. A. Map of Lake Nakuru, Kenya, showing detailed geographic features. B. Current surface area (April 2016) of Lake Nakuru (54.8 km2) as noticed from Google maps area calculator tool from the internet (Source: Adapted from Google maps, April 2016)

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 collecting water samples used to ascertain the levels of phytoplankton population densities, species composition, and conductivity measurements in the lake in 2014. The sampling sites were broadly ascertained by their accessibility as the lake was entirely flooded, 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 from 2009 to 2014. Samples were collected in sterile bottles and transported in a cool box to the School of Biological Sciences laboratory, Chiromo Campus, the 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. One replicate from each site was randomly selected to determine the phytoplankton cells concentration and species identification. 1 µL was taken from the bottle, 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 from 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 the January waterbird counts conducted jointly by the National Museums of Kenya and Kenya Wildlife Service.

Alterations in the lake levels. Data to ascertain the lake surface area and depth alterations were obtained from (Onywere et al. 2013) and the Kenya Wildlife Service records, respectively. Documentation of the lake surface area alterations 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). In contrast, monthly measurements of the lake's depth were collected from KWS. This had been ascertained from the readings of a staff gauge located at the lake center.

Physicochemical characteristics of water (phytoplankton concentration and conductivity). The physicochemical qualities of water (phytoplankton concentration and conductivity) for 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 center. Conductivity had been ascertained using a pH meter. The concentration of phytoplankton had been ascertained using the Sedgewick-Rafter counting chamber as described by Kimberly (1999).

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

Climate projection data sets. In this study, the projected alterations in near-surface temperature, rainfall, and evaporation for Lake Nakuru have been extracted from the Coupled Model Inter-comparison 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 forcing 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 2100, concerning 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 were then subjected to various statistical computations.

Homogeneity test. Most long-term climatological data records have been affected by several non-climatic factors that make these records unsuitable for comparison over long periods and between different stations. These relate to alterations affecting instruments, sites, procedures, observations, and data processing methods. These factors are caused by alterations in instrumentation, observation practices, location of the station, and formulae used for means calculation and changing the station's environment. While some alterations make critical discontinuities, others, particularly alterations around station environment, due, for example, urbanization, causes data biases which are gradually 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 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 the 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 consistency where cumulative rainfall and temperature data were 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 and its time of occurrence. Using the graphical method, this study plotted the annual means of rainfall, temperature, and evaporation data for 2000 to 2014. In addition, annual data means for lake depth, lesser flamingo population, conductivity, and phytoplankton levels for 2009 to 2014 were also plotted.

To ascertain the projected alterations in near-surface temperature, rainfall, and evaporation for Lake Nakuru, data extracted from the Coupled Model Inter-comparison Project Phase 5 (CMIP5) multi-model ensemble (IPCC Fifth Assessment Report (AR5) Atlas subset) models were plotted using the KNMI (2015) to analyze 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 growth or decline in a time series through a lengthy period. The trend in time series in this study, the graphical method was used to ascertain the past and current trends of climatic parameters (temperature, rainfall, and evaporation) and 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).

$$\sigma_M = \frac{\sigma}{\sqrt{N}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

Where, σM is the standard error of the mean, σ 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 imply 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 to measure 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 were ‘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 are associated and, if they are associated, the degree and direction of their correlation.

$$r = \frac{n\,(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n(\sum x^2) - (\sum x)^2]\,[n(\sum y^2) - (\sum y)^2]}} \quad \ldots\ldots\ldots\ldots\ldots \text{(2)}$$

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

$$t_{n-2} = r\sqrt{\frac{(n-2)}{1-r^2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(3)}$$

Where, n represents the length of the data used, *n-2* is the degree of freedom, *n-2* is the computed t-statistic, and r is the Pearson correlation coefficient.

The 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 model the population’s relationship.

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 the results of the homogeneity test. 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.

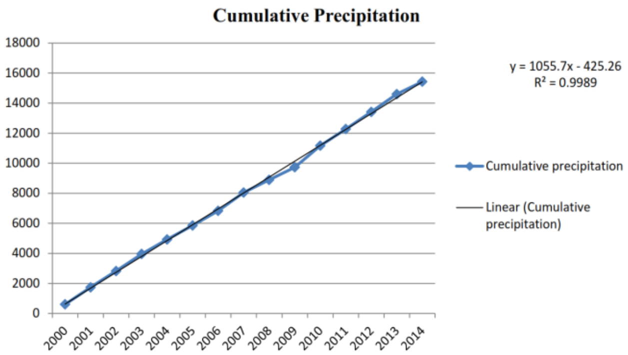


Figure 2. Single mass curve, cumulative annual rainfall

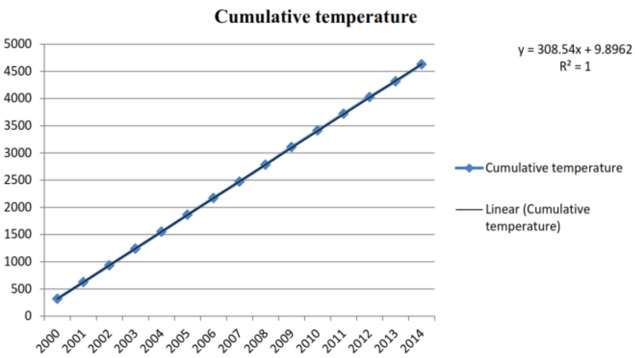


Figure 3. Single mass curve, cumulative annual temperature

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. Lake Nakuru basin's mean annual rainfall patterns marked variability with major rainfall intensification in 2000 to 2001 and 2009 to 2010, with the highest (120 mm) recorded in 2010 (Figure 4).

Trends in temperature patterns from 2000 to 2014. Mean annual temperatures have been on a lessening trend during the period 2000 to 2014, with the highest temperatures being recorded in 2000 (26.6ºC) and 2009 (27ºC) (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 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 risen from 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, leading 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 freshwater was added to it.

Alterations in the lake surface area

Lake Nakuru's surface area increased from 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 National 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 2009 to 2014 (Figure 11).

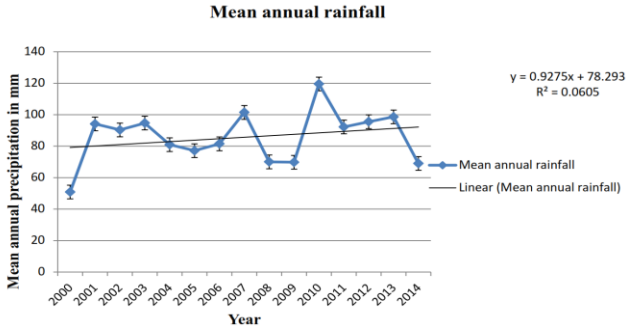


Figure 4. Mean annual rainfall patterns for the period 2000 to 2014

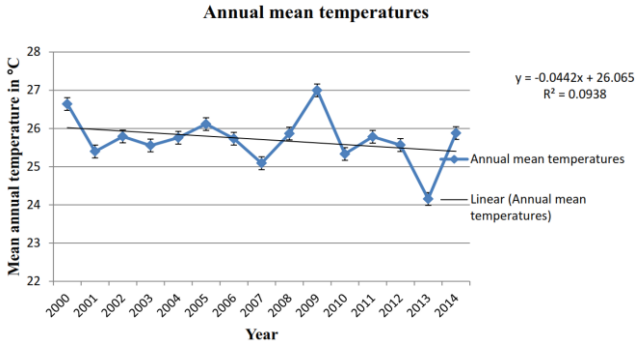


Figure 5. Mean annual temperatures from the year 2000 to 2014

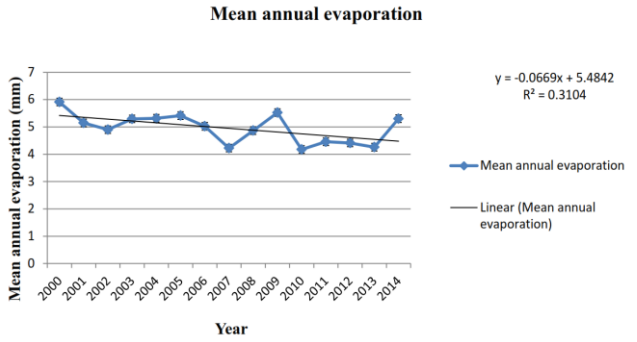


Figure 6. Mean annual evaporation patterns for the year 2000 to 2014

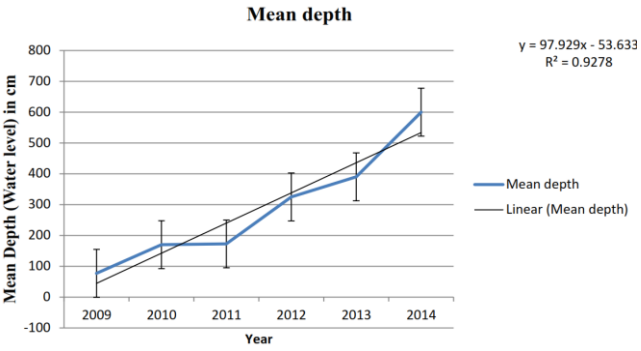


Figure 7. Alterations in the mean depth of Lake Nakuru from 2009 to 2014

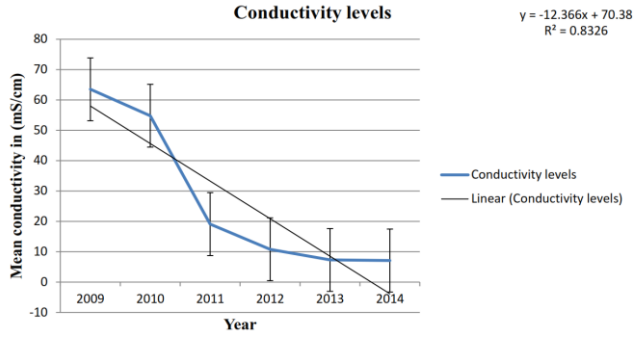


Figure 11. Trend in mean conductivity levels from 2009 to 2014 in Lake Nakuru

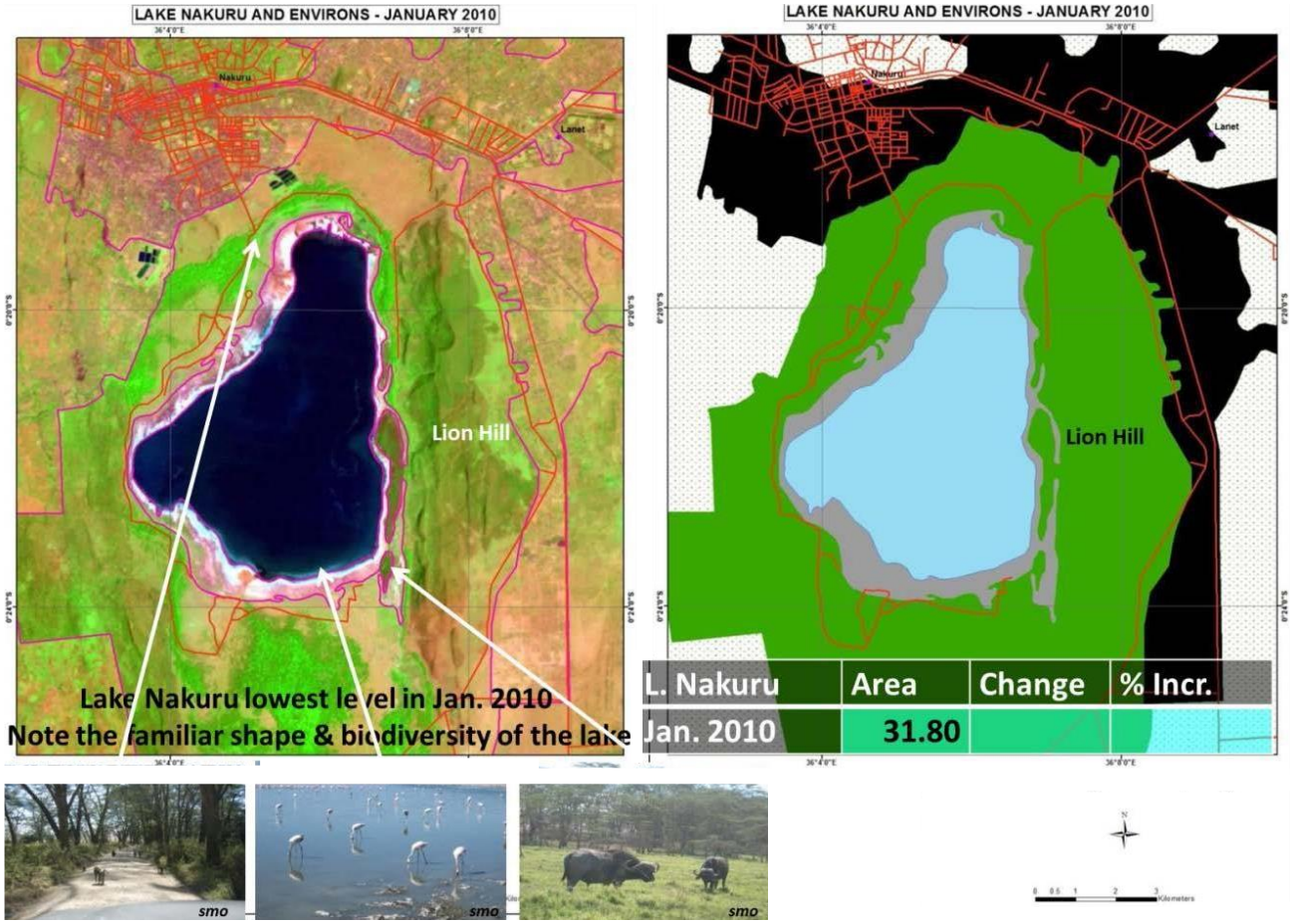


Figure 8. Alterations in the surface area of Lake Nakuru between January 2010 and 2013 (Source: Onywere et al. 2013)

This coincided with the beginning of the rains from 2010, as shown in Figure 4. The lake's declining conductivity could result in the 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 because as more freshwater was added to the lake, it lowered the conductivity of the lake. After all, freshwater has low conductivity, and the increment in water levels dilutes mineral concentrations.

Phytoplankton levels

The phytoplankton levels in Lake Nakuru were quite variable for 2009 to 2014, as shown by Figure 12.

Notably, however, a general reduction in the phytoplankton levels, coinciding with the rains' onset from 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 flamingo populations

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 reduced to 110 in 2012. This pattern follows that of lessening phytoplankton levels shown in Figure 12.

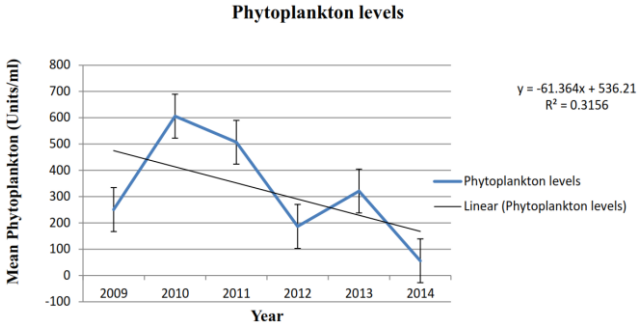


Figure 12. Trend in mean phytoplankton levels in Lake Nakuru from 2009 to 2014

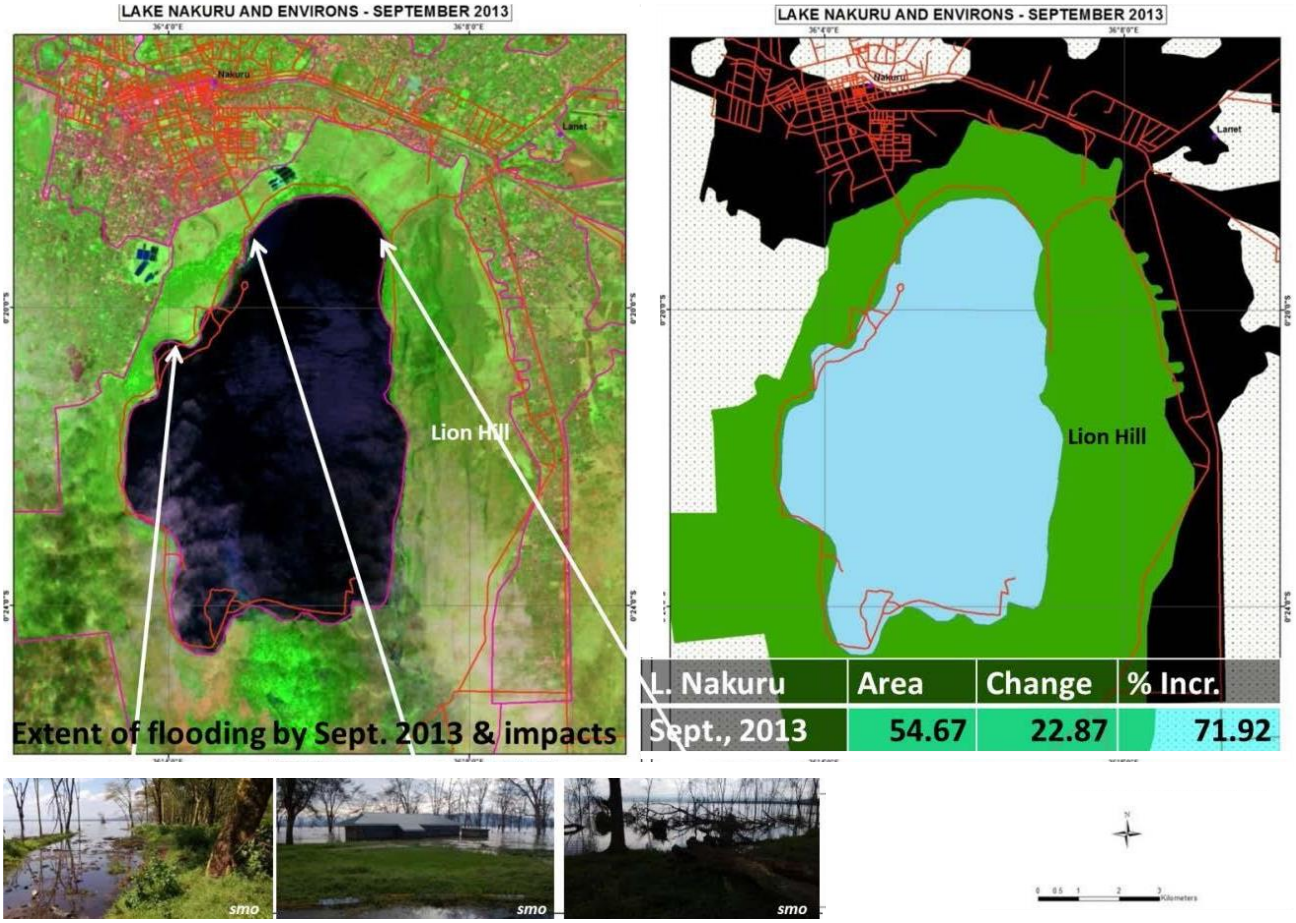


Figure 9. Lake Nakuru highest water level in September 2013 (Source: Onywere et al. 2013)

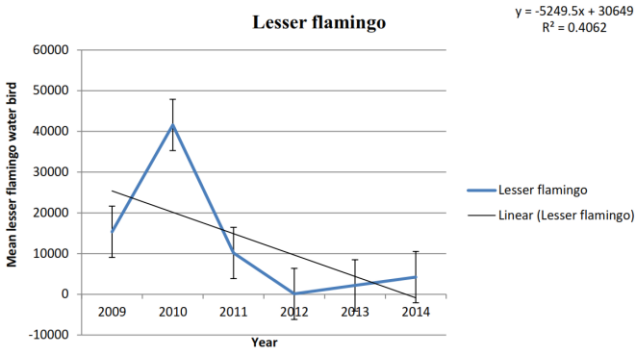


Figure 13. The trend in the number of lesser flamingos for the period 2009 to 2014

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

The findings in Table 1 showed a nonsignificant positive correlation between conductivity and phytoplankton (r=0.437, p=0.386). The results in Table 2 showed a notable positive correlation between conductivity and the lesser flamingo (r=0.767, p=0.075). The findings in Table 3 also showed a 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)
Conductivity in (mS/cm)	Pearson correlation	1	.437
	Sig. (2-tailed)		.386
	N	6	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
Conductivity in (mS/cm)	Pearson correlation	1	.767
	Sig. (2-tailed)		.075
	N	6	6

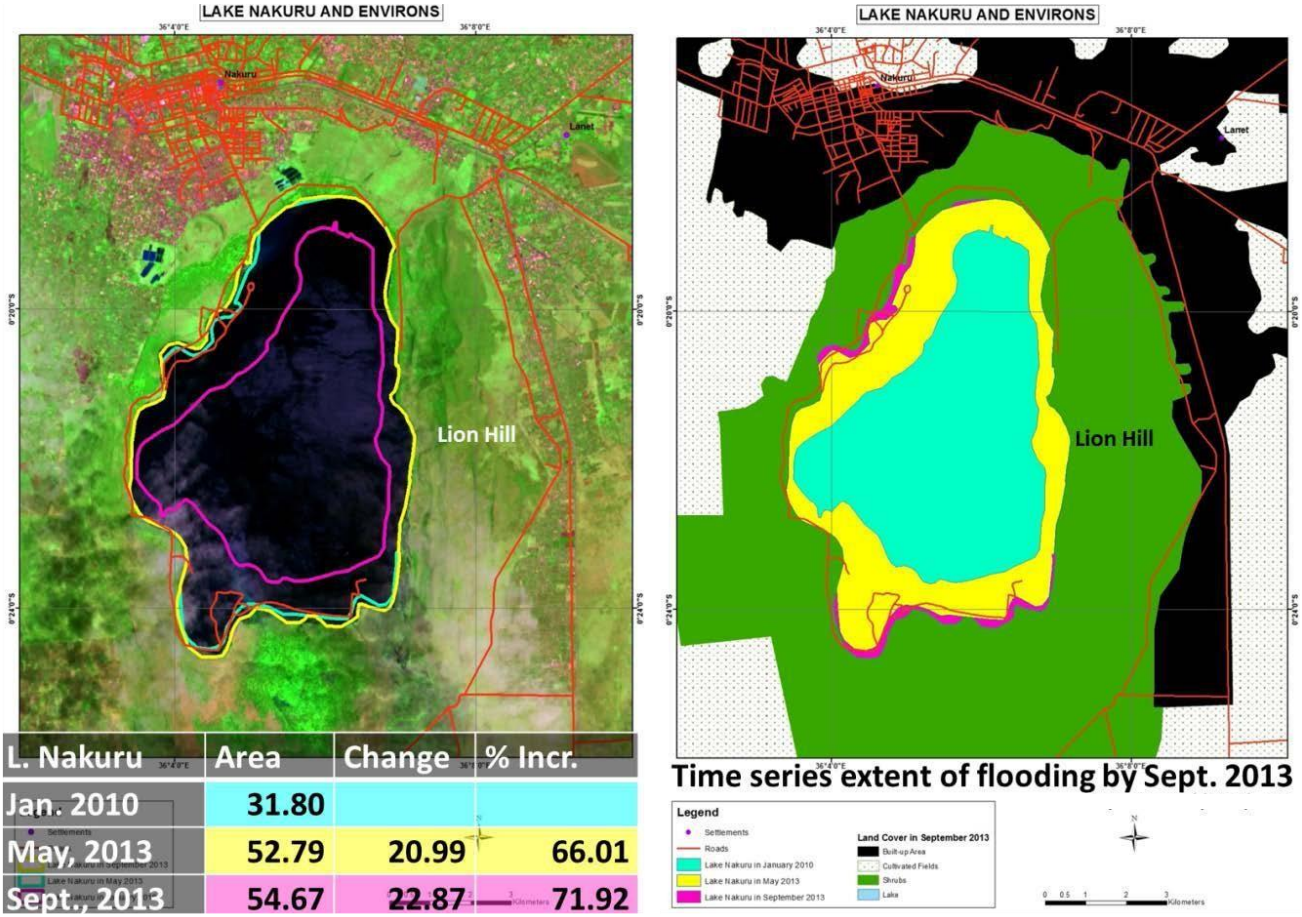


Figure 10. Time series extent of flooding in Lake Nakuru from a low area of 31.8 km² in January 2010 to a high of 54.7 km² in Sept 2013, a total increment of 22.9 km² (71.9%) (Onywere et al. 2013)

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 water bird	Phytoplankton (units/mL)
Lesser flamingo water bird	Pearson correlation	1	.731
	Sig. (2-tailed)		.099
	N	6	6

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

Lake Nakuru's future climate scenarios comprising near-surface temperature, rainfall, and evaporation were plotted for 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 the baseline period 1971 to 2000 are propounded in Figures 14 and 15, respectively.

Temperature projections indicate an accruing trend with a 1.2°C increment for 2071 to 2100 mean alterations for RCP2.6 (Figure 14), whereas there is a 4.8°C increment for 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 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 2017 to 2100 relative to the baseline period 1971 to 2000 are shown in Figures 16 and 17, respectively.

The rainfall projection from RCP2.6 and RCP 8.5 shows a 10% and 20 % increment in rainfall for 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

the baseline period 1971 to 2000 are shown in Figures 18 and 19.

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

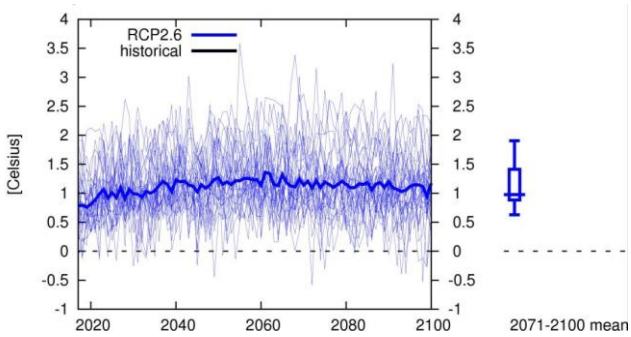


Figure 14. Near-surface temperature projection for RCP2.6 for the Lake Nakuru area from 2017 to 2100 shows a 1.2°C increment for 2071 to 2100 mean alterations

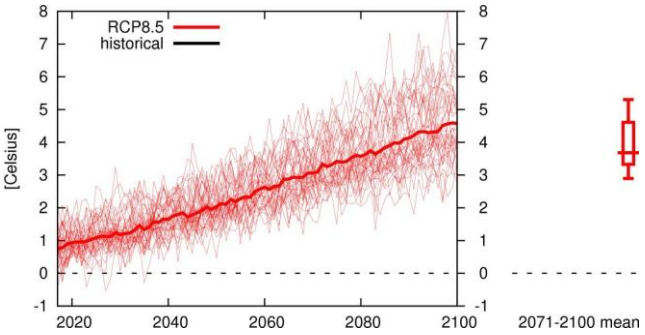


Figure 15. Near-surface temperature projection for RCP8.5 for the Lake Nakuru area from 2017 to 2100 shows a 4.8°C increment for 2071 to 2100 mean alterations

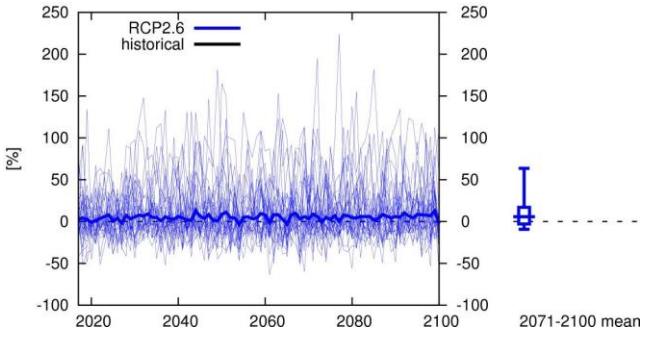


Figure 16. Rainfall projections for RCP2.6 for Lake Nakuru

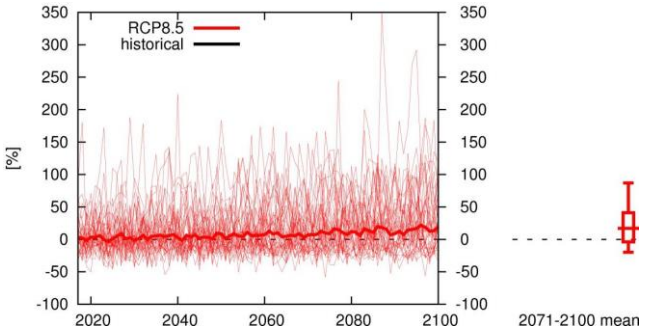


Figure 17. Rainfall projections for RCP8.5 show a 20% increment in rainfall in the Lake Nakuru area for 2071 to 2100 mean alterations

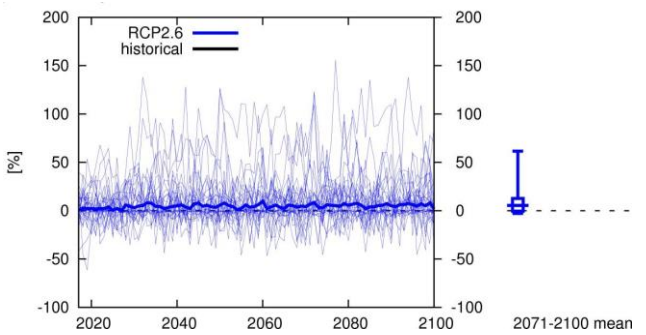


Figure 18. Relative evaporation alteration for RCP2.6 for the Lake Nakuru for 2071 to 2100

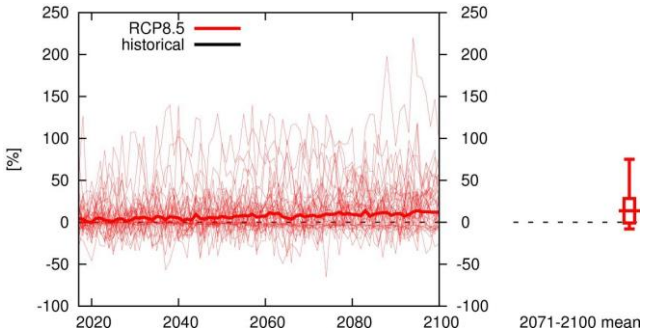


Figure 19. Relative evaporation alteration for RCP8.5 for Lake Nakuru

Discussions

As drawn in Figure 4, it can be concluded that rainfall has increased since 2010, and this may have significantly affected the accruing water levels in lakes, as shown by the images obtained from (Onywere et al. 2013). Figure 7 also depicts that the lake's depth 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 and direct rainfall to lakes.

Figures 5 and 6 depict that temperature and evaporation have been rising 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 rising from 2007 to 2009, which increased the ability to water retaining of the atmosphere, thus the beginning of rain in 2009.

According to the IPCC (2007), climate change is clearly more and easily accessed by temperature. However, atmospheric moisture changes, atmospheric circulation, and rainfall are also ascertained because the overall climate system is generally influenced. The capacity to withstand atmospheric moisture increment as the temperature becomes higher at a rate of about 7% per °C (Trenberth et al., 2003). Collectively, changes in the hydrological cycle are influenced, particularly 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 time 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 significantly affect endorheic lakes that may be extreme, resulting in severe algal biomass accidents and significant changes in community composition, as has been noticed in Lake Nakuru.

As more water is added to the lake, it liquidizes the mineral concentration, thereby lessening the lake's electrical conductivity. Freshwater has low conductivity. According to the study in Figure 11, the conductivity level began to decline in 2010, after 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 profoundly affect 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 biomass level that serves as food for the main feeder. The blue-green algal species, *Arthrospira fusiformis*, is one such species, and it is the leading food of the lesser flamingos. Thus, when the level of conductivity of the lake lessened, the rate of phytoplankton in the lake also reduced 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, it suggests that phytoplankton's denseness 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 shown in Table 3, confirms that in saline lakes, the distribution of lesser flamingo is affected by the availability of feed.

Figures 14 to 19 show an increment in temperature, rainfall, and evaporation for 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 lake's average depth 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. 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 lessen the level of conductivity in the lake, indicating the lake's increased concentration and dilution cycle 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.

Because the lake area has a negative rainfall/evaporation deficit, the rising 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 could 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 affect the phytoplankton availability, ascertained by the chemical and physical traits of the lake. There were fluctuations in 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 relate to 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 the population dynamics of the lesser flamingos may be affected by using the 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.

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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, Ong'amo GO, Ndegwa P. 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 8: 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 the primary transmission of RVF viruses. The high mobility of adult mosquito species has rendered interventions targeting their behavior ineffective. Thus, interventions that target immature stages an 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 quinquefaciatus*, *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 ($\chi_2^2 = 127.47$; 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 riverbanks, where the water was clear and vegetation was present. Stepwise regression analysis revealed that the 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 in the low land areas and 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 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 *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 result from 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 related to the El Niño phenomena. Secondary transmission in epidemics is mainly by female *Culex* mosquitoes and biting flies (Swanepoel et al., 2011; El Vilaly et al., 2013). From 2006 to 2007 Kenyan epidemic, 684 cases were reported, including 155 human deaths (23%). Among the 684 cases, about 183 were in the rift valley (WHO 2007), 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 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). Therefore, it is essential to continuously monitor changes in the diversity and distribution of these vectors to prevent 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 about 3,500 km^2. It lies in an agro-pastoral zone within Baringo County, Kenya. The temperature range is between 24° in the cold season and 30° degrees in the warm season. The 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 the google earth android application. The scores were selected based on the availability of potential larval habitats and accessibility. A handheld GPS receiver recorded each point's coordinates and elevation (Garmin, model *e*-Trex 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 into 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. At the same time, the high altitudinal points included: Kurget, Talai, Kaplewa, Kaptimbor, Borowonin, 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. The 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, it was classified as turbid. The observation was done directly in the habitat in habitats too shallow for using the 100 mL container.

A sampling of mosquito larvae from aquatic habitats

Every two weeks, sampling for immature mosquitoes was carried out 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 method could minimize the chances of immature mosquitoes swimming to the bottom of the habitat. The dipper was lowered gently at an angle of 45^0 so that collection was done 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.

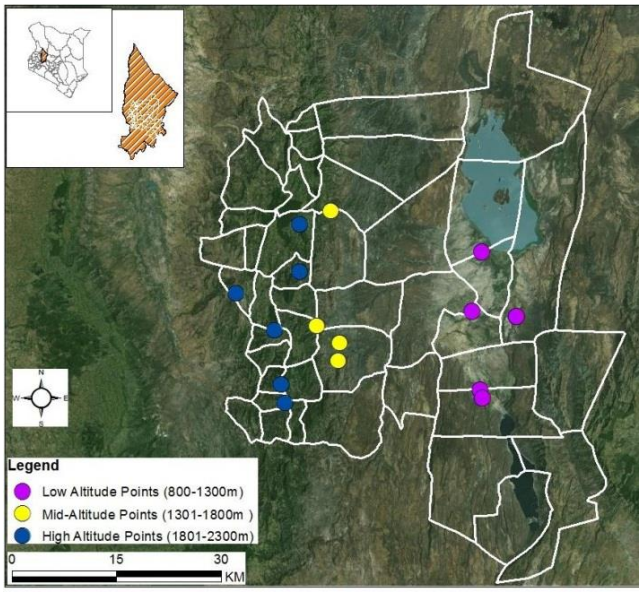

Figure 1. Map of Baringo County, Kenya, showing the study area and sampling points grouped into the three altitudinal classes.

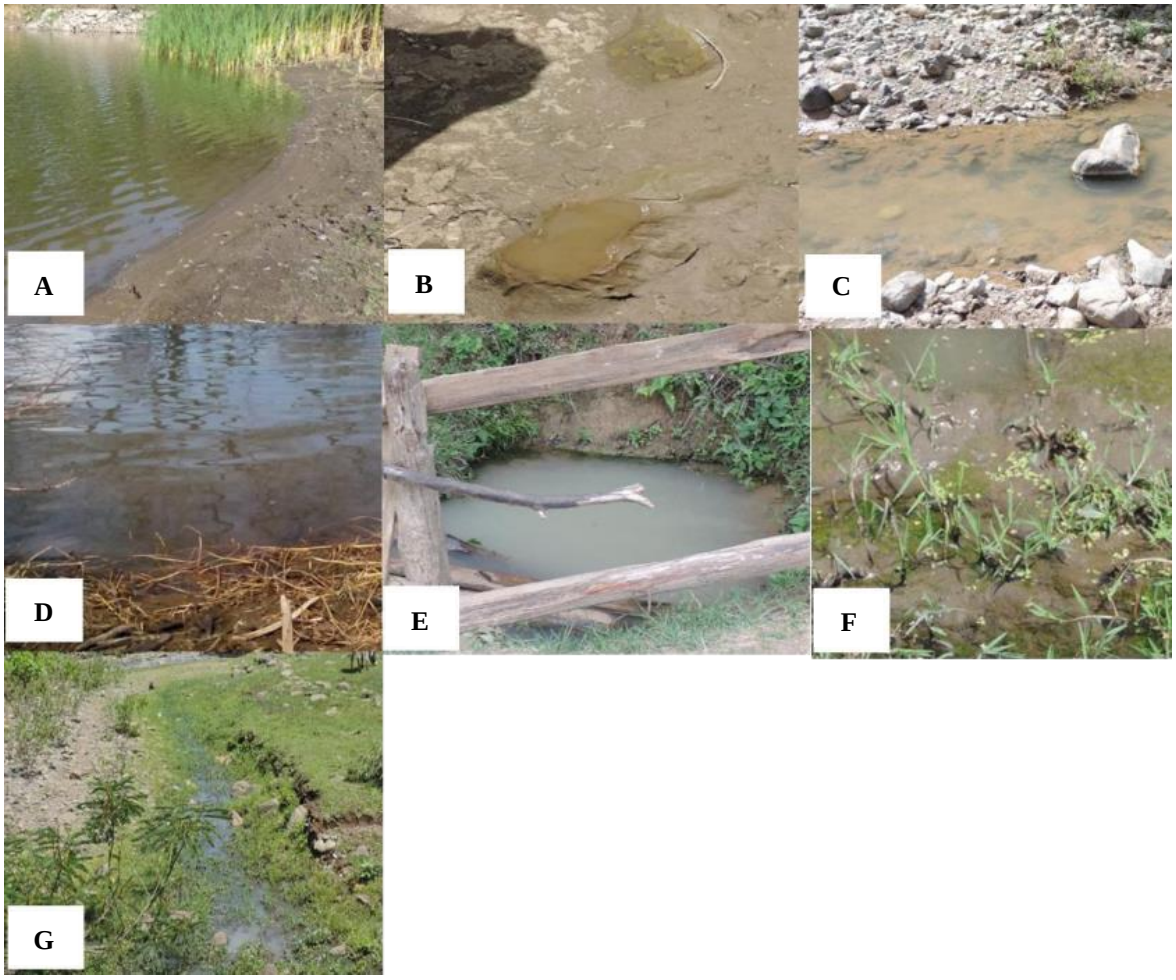

Figure 2. Images of different aquatic habitats: dam margin (A); animal hoof print (B); stream bank (C); Lake Flood zone (D); water pit (E); mash (F); spring bank (G)

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

Figure 4. The collection cup contained immature mosquitoes (A), Algae in a habitat (B)

After collection, the immature mosquitoes were transferred into a sealable collection cup using a plastic pipette, 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 indicates the point and date of the collections 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-quarters full of water from the source habitat containing algae, and loosely sealed to allow air in and out (Figure 4). To develop the fourth instar, they were left at room temperature (an average of 29^0C during the day) to develop the fourth instar. Each cup contained not more than 12 larvae. A week before the first collection, an experiment showed 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 organized for that species 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. The chi-square test made a comparison of mosquitoes collected in different altitudinal classes. A 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. Concerning 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 the 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. tigripes, An. coustani, An. gambiae* and *Ae. taylori*) between 1801 m and 2300 m (H' = 0.9836). Ofall the identified species, only seven (*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 (e^H'/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 (χ_2^2 = 127.47; *p*-value < 0.001). There was, however, no variation in the total number collected among the different altitudinal classes (χ_2^2 = 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, 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 the 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).

Culex quinquefaciatus was the most abundant mosquito species in the entire study area and *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 were only in the altitudinal class range between 1801 m and* 2300 m. It was also the least abundant in this altitudinal class range (Table 1). Further analysis showed that none of the *Culex* species significantly correlated with altitude (p>0.05; Table 2).

Anopheles species

The distribution of *Anopheles* species in the altitudinal class ranges were *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; *An. pharoensis* (205.0), An. coustani (56.3) and *An. gambiae* s.l (39.3) between 1301 m to 1800m; *An. 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_{32} = -2.50; p = 0.02; Table 2). Compared to *Culex* and *Anopheles* species, it was the second most abundant species after *Cx. quinquefaciatus*. *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_{32} = 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; ß = 2.38; t = 2.256; p = 0.0343). Habitat form significantly affected the number of *Cx. tigripes* (spring bank; ß = 3.951; t = 2.403; p = 0.0251), *Cx. annulioris* (Hoof print; ß = 27.2; t = -2.195; p = 0.039) and *An. pharoensis* (Marsh; ß = 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_{32} = -2.07, df = 32, p = 0.05: Table 2).

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

	Total abundance		
Species	**800-1300 m**	**1301-1800 m**	**1801-2300 m**
Culex pipiens	206.3	91.9	175.0
Cx. quinquefaciatus	886.6	409.0	1684.2
Cx. annulioris	76.0	126.5	130.0
Cx. 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
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 (ß = -0.5168; t = -2.617; p = 0.0157), Water pit (ß = -0.498; t = -2.345; p = 0.0284) and presence of vegetation (ß = 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

	Correlation to altitude			
Species	**r**	***T***	***Df***	***P***
Aedes taylori	0.32	1.91	32	0.07
Anopheles coustani	-0.24	-1.43	32	0.16
An. fenestus	-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. quinquefaciatus	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**	**Number of species**	***H'***
1334	Riverbank	Clear	Present	Present	7	1.721
1457.79	Dammargin	Turbid	Present	Present	5	1.467
1450.17	Springbank	Clear	Present	Present	5	1.466
1019.81	Marsh	Clear	Present	Present	5	1.458
1457.79	Dammargin	Clear	Present	Present	5	1.271
1925	Dammargin	Clear	Present	Present	4	1.219
1450.17	Springbank	Clear	Present	Present	4	1.203
982.93	Lakemargin	Clear	Present	Present	4	1.193
1323	Riverbank	Clear	Present	Present	5	1.123
987.2	Floodzone	Turbid	Present	Present	3	1.048
982.93	Hoofprint	Clear	Present	Present	3	0.9802
1019.81	Marsh	Turbid	Present	Present	4	0.9764
987.2	Floodzone	Clear	Absent	Absent	3	0.9743
983.24	Lakemargin	Clear	Present	Present	3	0.9103
2212	Springbank	Clear	Present	Present	6	0.8957
1015.24	Marsh	Clear	Present	Present	5	0.8025
983.24	Lakemargin	Clear	Present	Present	4	0.7834
987.2	Floodzone	Clear	Present	Present	3	0.6883
1457.79	Dammargin	Clear	Absent	Absent	2	0.6735
987.2	Floodzone	Turbid	Present	Present	2	0.672
2140	Waterpit	Clear	Present	Present	2	0.6555
999.7	Hoofprint	Clear	Present	Present	2	0.6269
999.7	Hoofprint	Clear	Present	Absent	2	0.6211
2177	Waterpan	Turbid	Present	Absent	2	0.5196
1837	Dammargin	Clear	Present	Present	2	0.518
2177	Waterpan	Turbid	Present	Absent	3	0.4769
2140	Waterpit	Turbid	Present	Present	4	0.4699
999.7	Hoofprint	Turbid	Present	Present	2	0.3365
2212	Springbank	Turbid	Absent	Absent	1	0
2179	Waterpit	Clear	Absent	Absent	1	0
2179	Waterpit	Turbid	Absent	Absent	1	0
2179	Waterpit	Turbid	Absent	Absent	1	0
999.7	Hoofprint	Clear	Absent	Present	1	0
987.2	Floodzone	Turbid	Absent	Absent	1	0

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 1999). Primary infections of RVF result from floodwater *Aedes* species, which are considered 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 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 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 results from their ability to feed on 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 of which Baringo, currently Baringo County, was part (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* were the least abundant. *Culex quinquefaciatus*, apart from being among secondary vectors of RVF in epidemics in Kenya (Sang et al. 2010), 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 cannot 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 indicates 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 (Hoogstraal et al., 1979). The laboratory test of *Cx. pipiens* strains have also shown that apart from being susceptible to the 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 primary 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. 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 the 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 indoors and is exophilic (Aniedu 1993). This might be the reason for its success since interventions in Baringo County mainly involve using insecticide-treated bed nets and pyrethrum spraying inside houses. These affect indoor bitters. *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 coustani* 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, habitat diversity negatively correlated with altitude, indicating that habitat diversity reduced as height increased. None of the Culex species showed any significant interactions on the interaction between species in a habitat. However, there were significant interactions between *An. funestus* and *An. coustani*. *Anopheles pharoensis* showed substantial interactions with *An. coustani* 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 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 with clear water and vegetation would be the most probable culprit for larvae breeding.

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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 8: 33-41.* The Wetland environment is unique with unique biota that includes insects. Insects serve as indicators of environmental health. Nevertheless, the recent spate 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 is positively correlated with insect diversity and abundance. Results of a survey of selected communities showed that the majority of the residents had a low level of education with less appreciation of issues involving the environment, including pollution. The majority of people within the surveyed communities could not 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 increase 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 that includes a large variety of landforms. Some cold climate wetlands are unique and thus have no tropical equivalent, and vice versa. The tundra and mangroves, for instance, are unique to the temperate and tropics, respectively.

Wetland resources comprise 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: 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 function (Samways et al. 2010). They can inhabit all conceivable habitats from the pole to the equator and occupy more trophic niches than the primary producers' level (Resh and Carde 2003). Insects are 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 and reflect the variety of plant resources available (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, thus exerting little or no pressure on insect species that act as fish prey. The absence of many fish and other insect predators within wetlands creates suitable habitats which enable insects to survive and persist, especially in swampy areas.

Insects are primarily 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, they also constitute a significant portion of the human diet in some parts of the world. Rare insects are sometimes used as indicators of endangered mammal species. Despite that, insects and such related indicator species are given little attention despite their importance in the overall ecological balance (Constanza et al. 1997).

This study investigated insect diversity of wetlands in three wetland environments related to human activities. We also investigated the impact of anthropogenic activities on wetlands within the study areas using the relationship between pollution and insect diversity in these wetlands. The aim was to use key insect species' presence or absence and abundance as proxy measures for degradation or otherwise of the wetland environments within the study areas. We hypothesize that some insect groups' presence (diversity and abundance) 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 water birds, 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 Ghana's Coastal Savanna Vegetation Zone, with a characteristic bimodal rainfall distribution and a low mean annual rainfall of about 854 mm. According to Gordon and Cobblah (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 short dry season from August to September. Mean annual temperature ranges from 24ºC in August to 29ºC in March, with a relative humidity range of 75-80% (Gordon and Cobblah 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, this area's previously diverse fauna, including mongooses, has 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 (Ntiamoa-Baidu and Gordon 1991; Ryan and Ntiamoa-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 5º 36.5‟ N and 5º 38.5‟ N and between the longitudes 1º 30' W and 2º 30' W. The district stretches from Madina to Oyarifa on the west and the Aburi highlands in the north. An approximate north-south line bounds it on the east, which also defines the western boundary of Tema (Biney 1995b). The surface area is 2.7 $km^{2,}$ and its catchment area covers a total area of 350 $km^{2,}$ although the active catchment area is 127 km^2 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 24.7ºC in August to a maximum of 28.1ºC in March. The lagoon and its neighboring wetlands have been labeled one of Ghana's five coastal Ramsar sites (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 of birding in the morning or afternoon at Sakumo between October and April should produce upwards of 80 species (Ryan 2005, Ntiamoa-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 5º 33′0 N and 5º 36′20 N and stretches between longitude 0º 9‟30‟‟ W′and 0º 7′10‟‟ W (Figure 1. C). The catchment area occupies almost 47.391551 ha. The Kpeshie lagoon is less than 1km^2 in surface area, and it is situated along the outskirts of La, a peri-urban township. The Municipal Assembly shares 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 lagoon's stretch, affecting 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 inter-site distance was 50m, and the largest was 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 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 that are not likely to be drawn to baits or light and assembled with brightly colored netting. Intercepted insects fall into the bottom trays containing 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 comprises 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 trapped mainly by this method. Individual traps within areas were separated 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 edge. 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, mainly between 8:00 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 major towns/settlements where water samples were collected

using purposive sampling and non-probability techniques. A total of 280 questionnaires were administered in Winneba, Sakumono, and Kpeshie. An effort was made to interview women and men equally in each locality.

Interview

Interviews were conducted by interacting with some 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 enveloped and labeled for further identification. All insects were identified to a 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 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 three sites combined. Muni-Pomadze recorded 1,883, Sakumo wetlands recorded 1,530, 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% during the dry season, respectively. Meanwhile, Hymenoptera and Hemiptera were dominant with 25% and 19% relative abundances, 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 analysis groups differed significantly ($p>0.05$). The abundance of insects did not vary much between Kpeshie and Sakumo, but Muni-Pomadze was relatively less than Kpeshie and Sakumo. However, there was a significant difference in the relative abundance of insects sampled during the dry and wet seasons. At all sampling sites, the number of individual insects collected in the wet season was higher than in the dry season (Table 1). Hymenoptera was the richest order in both seasons at Sakumono, 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 and 12% in the dry seasons. Muni-Pomadze recorded a total relative abundance of 84% in the wet and 15.6% in the dry seasons.

Species richness and diversity indices

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

Observed species richness

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

Social survey: Respondent information

Sex

The questionnaires were administered in Sakumono, 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 Sakumono 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
Thysanopter	18	58	43	2.2
Mecoptera	0	4	5	0.1
Isoptera	10	21	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	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
Keshie	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

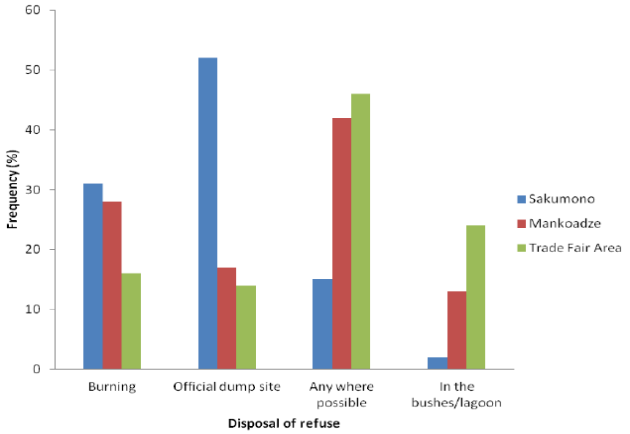

Figure 2. Mode of disposal of refuse

Educational level

Table 4 summarizes 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; whiles 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 Hymenoptera was the highest number and far more varied order recorded at all three sites, with Kpeshie being the most varied.

The abundance and diversity of insects at a specific habitat depend on various 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 (Struhsaker 1998; Nummelin 1996). The same factors likely accounted for the differences in species composition within the area. Insect numbers from Kpeshie were higher than those at Muni-Pomadze. This may probably have been due to the diversity of plants and 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 Sakumono and Muni-Pomadze. Ants, which constitute most 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 insects. Many cattle were observed grazing in and around the vast grassland areas around the lagoons. Sakomono 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 Culicidae. The many heaps of waste provided perfect breeding sites for these mosquitoes. Many dipterans such as the Muscidae and Calliphoridae thrive on decaying matter which was also in abundance. Simulidae were found where there were ripples created by the presence of giant 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). Even though 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, which 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, solid wastes quickly find their way into the lagoon. In contrast, liquid waste is discarded directly into the lagoon from drains from all the nearby settlements 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* (Culicidae), and *Simulium venustum* (Simuliidae).

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 temperature variations recorded in the dry and wet seasons were higher than 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 a 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 Sakumono and 17% for the La Trade Fair area (Kpeshie), respectively, with Mankoadze having only 9%. Mankoadze observed the highest number of middle and JHS leavers with 25% respondents followed by 24% of people for Sakumono and 18% for La Trade Fair. Sakumono and La Trade Fair areas had the highest number of respondents with vocational, commercial, and technical educational status. It is evident 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 entering the lagoon has its sources from communities within Burma Camp, La, Tebibiano, Teshie Camp, Africa Lake (all communities in the catchment area), and 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 many 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, riverbanks, 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. There was indiscriminate human fecal matter scattered all over most 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). They reported that sanitation is the least managed problem in developing countries, with much pollution coming from domestic rather than industrial sources. The neglect of good hygiene practices makes it extremely difficult to control 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 due to 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 has 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 humanitarian conditions in communities where these wetlands are located. This is further aggravated by the lack of decent toilet facilities and managed to refuse damping 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 local governments' lack of or unwillingness to manage or protect the three studied wetlands, two of which are designated Ramsar sites. Lousy farming practices, improper domestic and industrial waste disposal, and lousy fishing practices were identified as the primary sources of pollution in these wetland environments. If the trend is not arrested, there are indications of further destruction of these wetlands and other such wetlands in Ghana, 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.

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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 garieoinus. *Bonorowo Wetlands 8: 42-50.* Bacteria under the family Flavobacteriaceae (also called Flavobacteria) are important pathogens of fish, people, many other animals, and plants in this study. 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 119 fish from 19 farms and were dissected aseptically to sample internal organs. The samples were inoculated onto Shieh 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 characteristic colon similarity. One group selected one isolate for 16S rRNA gene sequencing and identified using the EZbiocloud.net ID software. Phylogenetic analysis of selected isolates was performed using the 16S rRNA gene sequences in BioEdit and MEGA 7.0.2 software. Based 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; *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 subculturing. Phylogenetic analysis indicated that *M. marinus* isolates grouped with other *M. marinus* isolates from gene bank with significant intra-species diversity, 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 garieoinus*, 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, an increase in aquaculture is accompanied by an increased risk of diseases. Earlier it was observed during 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 a ruinous effect on the wild and farmed fish stocks. Flavobacterial disease eruptions are infamously challenging to avert and control, even though much 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, making 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 that Uhas a high growth rate in aquaculture (MAAIF 2004). Information about Flavobacterial diseases in Uganda is not well documented, but several undocumented cases (unpublished, NAFIRI, 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. 2012 a,b; Loch and Fasial 2014), could be one of the indications that Uganda will at one time face the same problem. Therefore, it is important to proactively study species prevalent in the country and 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 based 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, and spleen. These were homogenized by cutting into smaller pieces using a sterile surgical blade and then inoculated into Shieh 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 based on colony morphology, Gram staining, standard biochemical tests, and their consistency. All yellow bacterial colonies were considered for the study. Shieh agar and Shieh broth were made for bacterial growth as in the table in 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 a light microscope (magnification, x 100). Other biochemical tests included; the 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 Biomerieurix were also used at Makerere University and the Norwegian University of Life Sciences (NMBU) as screening tests to identify isolates before further sequencing.

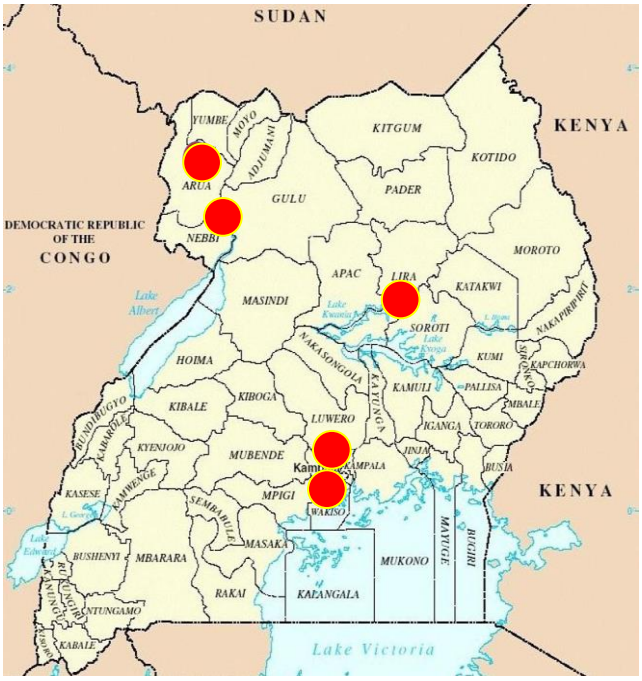

Figure 1. Map of Uganda and study area

Molecular identification of Flavobacteria

The isolates were preserved on Shieh 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 Merck KGaA Germany and the suspected Flavobacteriaceae colonies were divided into eight groups based on colony morphology similarity (based 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 a QIAamp DNA mini kit (Qiagen). The manufacturer`s protocol was followed as stated in appendix 2, and all spin steps used a benchtop Mini spin 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'-GGTTACCTTGTTACGACTT-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 $MgCl_2$, 20MM $(NH_4)_2SO_4$), 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 icycle (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 the 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 were 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 a QIAquick Gel Extraction kit (Quiagen) following the manufacturer's instructions as stated in appendix 2. The Purified PCR was quantified, and quality was 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 were summarized and stored in Microsoft Excel version 10. BLAST searches were done online to get similar sequences from the NCBI website's gene banks. The obtained sequences from isolates in this study were edited using bio edit and aligned with those retrieved from gene banks using the *Claustal W* algorithm in MEGA version 7.0 software. The alignments were used to construct a phylogenetic tree using the Neighbor-Joining method using the Kimura-2-parameter model. Identification of the sequences was also made 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 were encountered in the fish samples.

Both symptomatic and asymptomatic fish were sampled; some of the lesions encountered in the symptomatic fish included: hemorrhages on the 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 Silver carp	Lake River Underground Streams	5 farms (26.31%) with disease outbreak/ history	13 farms with only earthen ponds 3 farms with only cages
3 large scale farms	over 20 units for large scale	African catfish Tilapia			2 farms with tanks and ponds 1 farm with tanks only

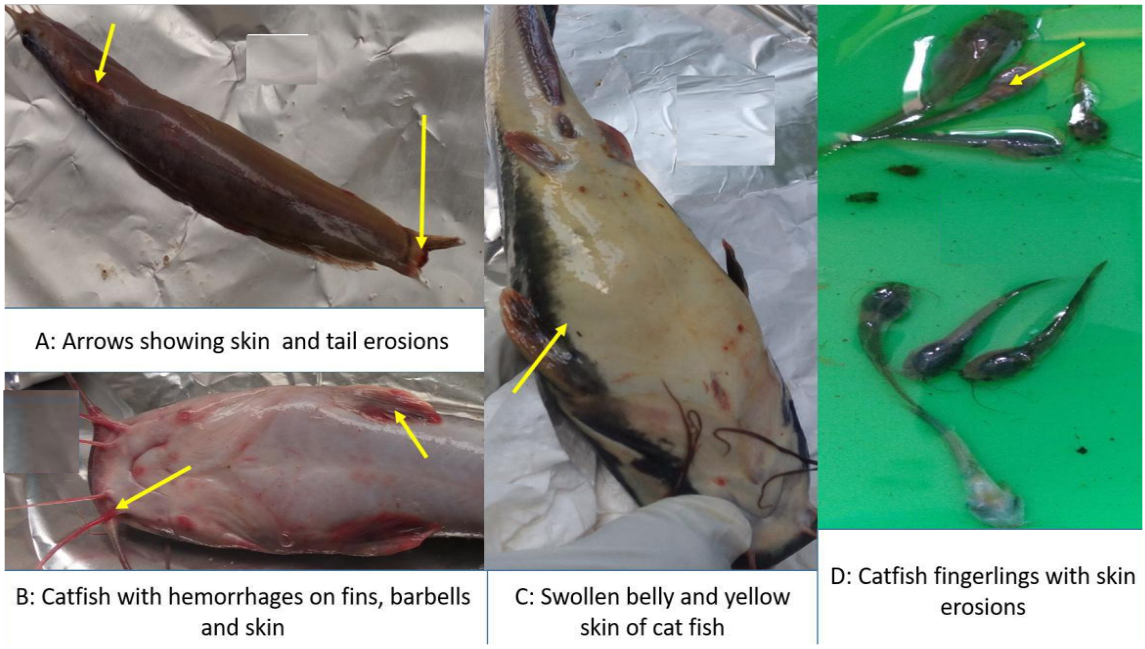

Figure 2. Lesions encountered on catfish

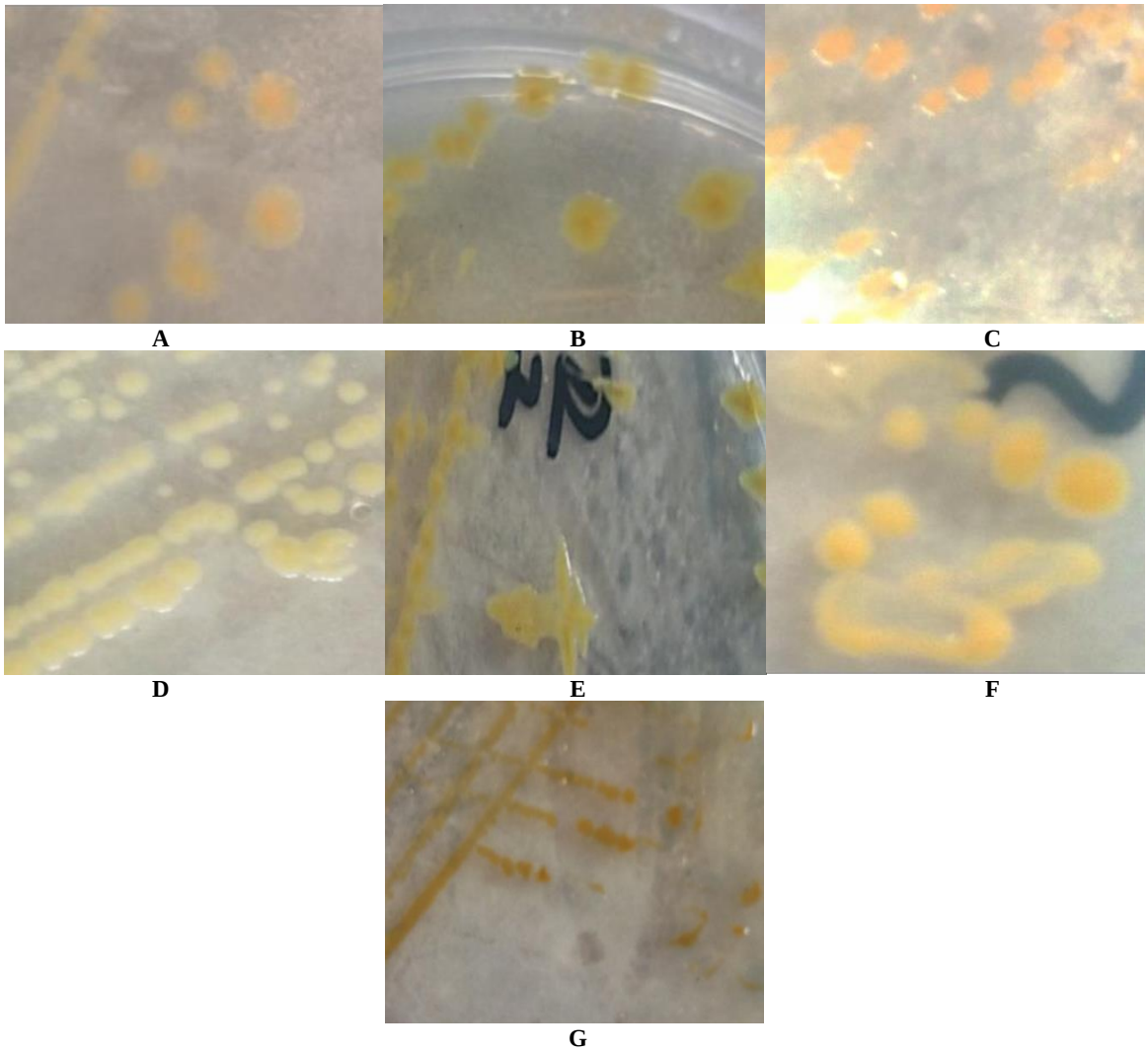

Figure 3. Colony characteristics of the study isolates. A. Isolate 1. Soft, sticky, bright yellow, flat, large size, smooth; B. Isolate 2. Soft, large, yellow, flat, gelatinous; C. Isolate 3. Yellow, medium size, flat, glistening; D. Isolate 5. Pale yellow, round, flat, medium size, shinny; E. Isolate 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 Shieh broth followed by streaking the broth on Shieh agar always gave fewer types of colonies (sometimes only one) per sample than direct streaking of the gill and skin swabs on agar. A total of 86 isolates were obtained from the 119-fish sampled, with colonies ranging from pale yellow, bright yellow, deep yellow to orange and had a fruity odor and 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 varying sizes ranging from small to large. These were grouped into 8 and one colonies per group selected. Figure 3 shows some of the chosen colonies for sequencing but missing the colony for isolate 4.

Biochemical test results

Biochemical test results for the sequenced isolates

The biochemical test results are summarized in Table 2. Some colonies produced H_2S, but after storage and sub-culturing and their TSI test did not give off H_2S.

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

Table 2. Biochemical test results of the sequenced isolates

Isolate	Catalase	Oxidase	Flexirubin Pigment	Congo red absorption	H_2S	Urease	Gelatinase	Indole production	Motility	Glucose fermentation	Gas off glucose	Sucrose fermentation
1	+	+	+	+	-	+	-	+	-	-	-	+
2	+	+	+	+	-	+	+	-	-	-	-	-
3	+	+	+	+	-	+	+	-	-	-	-	-
4	+	+	+	+	-	-	+	-	-	-	-	-
5	+	+	+	+	-	-	-	-	-	-	-	-
6	+	+	+	+	-	-	+	-	+	-	-	-
7	-	+	+	+	-	+	-	-	-	-	-	-
8	+	+	+	+	-	-	+	-	-	+	+	-

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	*Myroides* sp.	64
H	1	*Weeksiela* sp.	37
1	1	*Myroides* sp.	64
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, presence 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 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	-	+	+	+	+
	Conventional	-	-	+	+	+
B	API	+	-	+	+	+
	Conventional	-	+	+	+	+
3	API	-	+	+	+	-
	Conventional	Missing	+	+	+	-
C	API	-	-	+	+	+
	Conventional	-	-	-	+	+
D	API	-	-	+	-	+
	Conventional	-	-	+	+	+
E	API	-	+	+	-	-
	Conventional	-	+	Missing	+	+
F	API	-	+	+	-	+
	Conventional	-	+	+	+	+
1	API	-	+	+	-	-
	Conventional	-	+	+	+	+
G	API	-	+	+	-	-
	Conventional	-	+	+	+	-
H	API	-	-	+	-	-
	Conventional	-	+	+	+	-
I	API	-	+	+	-	-
	Conventional	-	+	+	+	-

Table 3. General biochemical test results of the groups

Representative sequenced isolate	1	2	3	4	5	6	8*
No. of isolates in the group	**14**	**3**	**30**	**13**	**1**	**1**	**2**
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 (-)	100 (-)	92.3 (-)	(-)	(-)	(+)
Sucrose fermentation	92.9 (-)	100 (-)	93.3 (-)	92.3 (-)	(-)	(-)	(-)

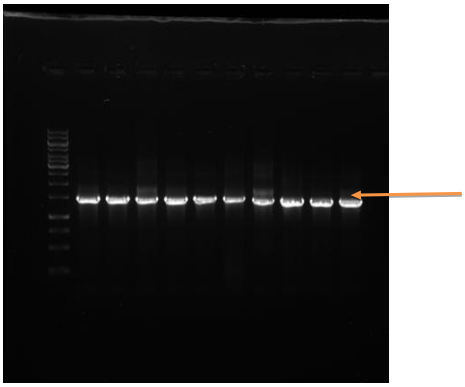

Figure 4. Electrophoresis results for the 16S rRNA gene

Identification and occurrence of the isolates

Identification of isolates using Ezbiocloud.net

The most familiar isolated species was *M. marinus. 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's* isolates) using Ezbioclod.net. These were isolated on 15 farms out of the 19 sampled farms. The least common species isolated were those closely similar to *M. odoramitimus*, with closet 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 *Clariaus 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 the extrapolation of the results of the sequenced isolates.

Phylogenetic analysis

Occurrence of flavobacteria on the farms

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: The Neighbor-Joining method implied the evolutionary history (Saitou and Nei 1987). The optimal tree showed the sum of branch length = 0.51734957. 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 the reference strain *M. marinus JS 08* compared to isolates 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 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 than isolate 8.

Table 6. Identification of isolates and their occurrence in fish

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

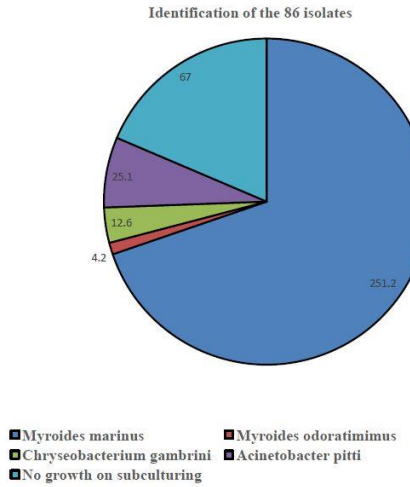

Figure 5. Identification based on the extrapolation of results of sequenced isolates

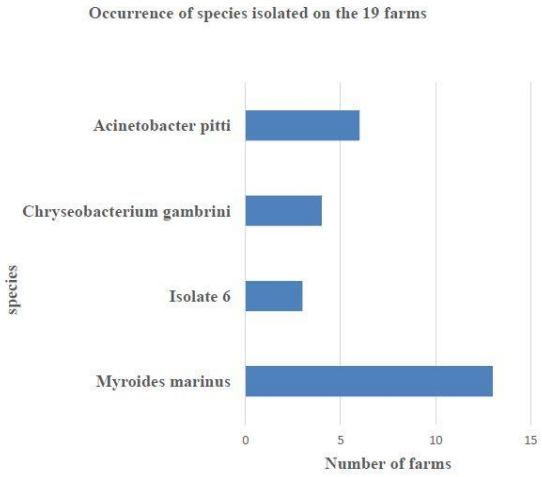

Figure 6. Occurrence of isolates on the selected farms

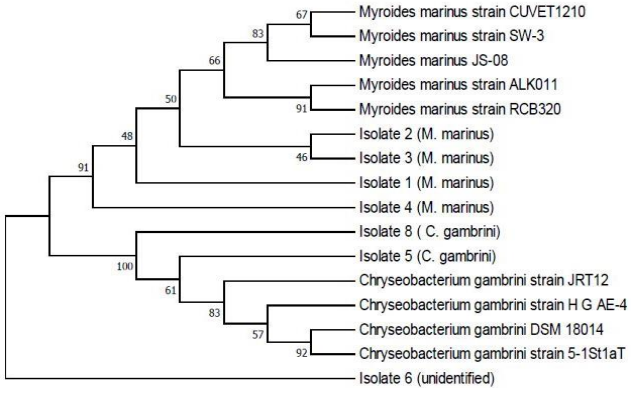

Figure 7. Phylogenetic relatedness of the isolates based on the 16S rRNA gene

The graphical views showing a 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. gambrini* JGI1096583 in the regions between 270 and 277, 978 and 996. Isolate 8 had more nucleotide differences to the reference strain than isolate 5.

Discussion

Flavobacteria are significant 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 to understand the Flavobacterial diseases better.

In this study, all the selected farms had at least one bacterium from the family *Flavobacteriacea* isolated, and some had more than one colony type of the isolates. Some of these Flavobacteria, like the *Myroides species*, can 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 subculturing 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 fish health importance (Loch and Fasial 2015).

Seven out of the eight selected representative isolates in this study were closely related to the 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 most familiar 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 getting water sources 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 based on similarity in the 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. Some isolates identified as the 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 the 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 the strains that were not well studied here. The fact that some of the isolates had a phylogenetic relationship and yet were found in different farms in different parts of the country could indicate a 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*, 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 the family Flavobacteriaceae.

Isolate 7, although with the colony and biochemical characteristics similar to Flavobacteria, was identified as *Acinetobacter pitti* 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 are insufficient 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 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 were 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 to identification by API kits and 16s RNA gene sequencing was not well studied here. However, both API kits and 16S RNA gene sequencing did not identify any major Flavobacteria. The API test results for isolates G and I at 64% identity gave a correct genus identification even though the percentage identity was still considered low. For isolates H and I, the percentage identification was below average; the identification was incorrect compared to sequence identification. The API results in this study generally had low percentage identities and were not reliable. Adley and Saieb (2005) compared biomeriueux API 20NE and Remel RapiD NF Plus in the identification systems of type strains of *Ralstonia picketti.* Only 29 out of 48 isolates were identified, and the API 20NE was considered inconsistent. However, the use of API kits (API NE and API ZYM) in a study by Farmer proved to be useful in identifying *F. columnare* (Farmer 2004). When used in this study, API NE kits could rule out *F. columnare* even though colony morphology on Shieh agar and biochemical tests suggested otherwise. The identification by API kits and 16s RNA gene sequencing was not well studied here. However, both API kits and 16S RNA gene sequencing did not identify any 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 Table 5. However, the number of samples tested and the number of the biochemical tests compared were too small to be reliable for a consequence.

Distinct findings were furnished in this study compared to those of the previous studies done in Uganda, which indicated a high incidence of *F. columnare* (Walakira et al. 2014). However, in this study, there is an increased occurrence of bacteria under the family Flavobacteriaceae except for *F. columnare*. There is a possibility that the presumed *F. columnare* in Walakira et al.'s (2014) study could have been different species under the o genera of the 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). There are few diagnostic reagents specific for the lesser-known fish-associated Flavobacteria organisms. Diagnosis is further made more difficult because 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 surveyed the incidence of *F. columnare* in wild and cultured freshwater fish species in Hungary. A total of twenty-five isolates from wild and cultivated freshwater fishes were identified as F. columnare 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 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 obtained 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, such as isolates 1 and 8, were recovered from catfish fingerlings (*Clariaus gariepinus*) that were reportedly experiencing abnormal mortalities for a week. Isolate 8 was identified as *C. gambrini.* Loch, in his study, stated that *Flavobacterium* sp. and *Chryseobacterium* spp. were an extensive cause of fingerling and fry mortalities in Michigan (Loch 2014). However, this case 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 Fasial 2015). The Original Flavobacteria known to be causing fish health issues were the *F. columnare*, *F. branchiophilum*, *F. psychrophilum*, but there are many other *Flavobacteriacea* 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. spartani sp*. nov. was rather more pathogenic (Loch 2014). Thus, it is important to study the pathogenicity of emerging Flavobacteria.

Some farmers reported poor growth of fish. This could be due to many other factors that could include but are 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. *Myroides marinus* was common in the selected farms in this study isolated on 13 farms which are 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. psychrophillum*) was identified in this study. The routinely used biochemical and morphological growth characteristics were insufficient to identify Flavobacteria. Phylogenetic analysis indicated that *M. marinus* isolates grouped with other *M. marinus* isolates from the gene bank. Although intra-species diversity was observed, a similar situation was observed with *C. gambrini* isolates.

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