Phytoplankton distribution along a salinity gradient in two Kenyan saltworks (Tana and Kurawa)

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Introduction
Saltworks are constituted of a series of interconnected ponds with increasing salinity from inlet to crystallizers. Natural seawater is drawn from a nearby creek or lagoon often by pumping into a large reservoir, and then allowed to flow by gravity through a series of shallow interconnected man-made ponds where evaporation fueled by wind and solar energy occurs (Korovessis and Lekkas, 1999). Since evaporation occurs in stages, ponds of narrow salinity ranges are created where different salts present in the original seawater precipitate based on their solubility. Low soluble salts such as calcium carbonate and sulphate precipitate in salinities below 200 ppt while sodium chloride, which is highly soluble, precipitates in the crystallizers (300–350 ppt) (Gongora et al., 2005).

The natural seawater brought into saltworks also brings with it inorganic nutrients and several marine organisms, which establish stable populations at different salinity levels in saltworks ponds. Among the organisms described in saltworks include phytoplankton (Dolapsakis et al., 2005; Elloumi et al., 2009; Evagelopoulos et al., 2009; Radhika et al., 2011; Chatchawan et al., 2011; Madkour and Gaballah, 2012; Devi et al., 2019), ciliates (Elloumi et al., 2006), halophilic bacteria and archaea (Casamayor et al., 2002; Oren, 2008), zooplankton (Mustafa et al., 1999), as well as macrobenthic invertebrates (Evagelopoulos et al., 2009) and the brine shrimp (Elloumi et al., 2009; Mohebbi, 2010). The consensus from most studies is that species richness, evenness and diversity declines with increasing salinity (Evagelopoulos et al., 2009). Abundance on
the other hand, has shown different trends for different organisms. In most studies, phytoplankton density and biomass have been reported to be highest in the high salinity ponds. Ciliates and most zooplankton except *Artemia* have shown a decreasing trend with increasing salinity in most studies (Elloumi *et al*., 2006; Radhika *et al*., 2011). The wide range of planktonic and benthic communities of marine organisms that develop along the salinity gradient constitute a biological system that plays an important role in the production of high-quality salt through preventing leakage of brine, improving solar energy absorption and water evaporation (Madkour and Gaballah, 2012).

Diversity and abundance of phytoplankton in saline lakes and saltworks has been an area of research focus by several scientists in the recent past. Phytoplankton sit at the base of marine food webs and act as primary producers where they convert solar energy into chemical energy. Zooplankton feed on the phytoplankton and pass the energy to higher trophic levels. In addition, phytoplankton produce oxygen and organic matter that support the flourishing of microbial communities in aquatic environments (Jakhar, 2013). The most common phytoplankton groups that have been described in saltworks include Dinophyceae, Bacillariophyceae, Chlorophyceae, Euglenophyceae, Raphidophyceae, Prymatesophyceae, Cryptophyceae, and Silicoflagellates. The most dominant among the groups identified have been Bacillariophyceae and Dinophyceae in ponds of salinities below 100 ppt, and Cyanophyceae and Chlorophyceae (*Dunaliella*) in salinities above 100 ppt (Tiffany *et al*., 2007; Evagelopoulos *et al*., 2009; Madkour and Gaballah, 2012). Despite the numerous studies conducted on phytoplankton diversity in saltworks, there is limited information on the diversity and abundance of phytoplankton in tropical saltworks.

Kenya is an important salt producing country in the East African region with over 5 large commercial saltworks companies and several artisanal salt farms operating along the Kenyan coast (KNCHR, 2006). The salt farms have been identified for integrated production of salt and *Artemia*. *Artemia* is an important live feed in the larviculture of marine fish (FAO, 2011). Initial inoculation of *Artemia* in Kenyan saltworks was done in the mid-1980s at a time when similar initiatives were undertaken in some Asian and Latin American countries (FAO, 2011; Ogello *et al*., 2014). Following successful post-inoculation colonization and establishment of stable *Artemia* populations in Kenya, efforts are underway to commercialize the integrated salt-*Artemia* farming model to generate enough *Artemia* cysts for the revitalised mariculture sector in the country, while at the same time improving overall revenues earned by salt producers. *Artemia* products are valued based on their nutritive value especially levels of essential highly unsaturated fatty acids, which are dependent on the diet of the *Artemia* (FAO, 2011). Primarily, *Artemia* feeds on phytoplankton present in the saltworks and this is a major determinant of *Artemia* quality. An understanding of the distribution and abundance of phytoplankton in Kenyan saltworks is important to inform efforts to improve the quality of *Artemia* cysts and overall biomass.

The present study seeks to determine the spatial distribution of phytoplankton along a salinity gradient in two Kenyan saltworks (Tana and Kurawa) using samples collected in March and September 2021. Sampling of phytoplankton was carried out at five salinity ranges (30-40, 50-60, 90-100, 150-160 and 190-200 ppt). It is expected that the information generated will benefit commercial and artisanal salt producers as well as policy makers to optimize *Artemia* production in saltworks.

**Materials and methods**

**Study area**

The study was conducted at the Tana and Kurawa salt farms, which are among the major salt producing companies in Kenya (Fig. 1). Kurawa saltworks is located in Kurawa area, Magarini Sub County, Kilifi County (2°44’3.00”S, 40°11’5.00”E) about 1 km off the Malindi-Garsen road at Kanagoni town. The saltworks covers an area of about 595.2 ha (KNHCR, 2006; Mumbah *et al*., 2017). Tana saltworks is located in Tana River County (2°41’38.00”S, 40°11’5.00”E) about 14 km from Kanagoni town and occupies an area of approximately 380 ha (Yap and Landoy, 1986). Salinity of water circulated in the two saltworks ranges from 33 ppt at the inlet to 300 ppt in the crystallizers.

**Sampling**

Sampling was carried out selectively in salt ponds of varying salinity ranges (30-40, 50-60, 90-100, 150-160 and 190-200 ppt). In situ measurement of water temperature, salinity, total dissolved solids (TDS) and conductivity was carried out using a handheld multi-parameter sensor (YSI Professional Plus, USA) prior to collection of water samples. The water quality data was collected from four different points in one pond. The first sampling was done in the month of March 2021 at Kurawa and Tana between 0900 h to 1200
For each pond, 40 l of saline water were filtered using a 20 µm plankton net of 30 cm diameter and the remnant was transferred to 250 ml bottles. This procedure was repeated twice for each pond sampled. The samples were immediately fixed with 1 ml of acidified Lugol’s solution to prevent predation by zooplankton and to preserve the collected microalgae cells. Samples were transported in a cooler box to the laboratory, received and stored in the dark at room temperature awaiting laboratory analysis. The same procedure was repeated during the second sampling in the month of September 2021. March is the peak of the dry season in Kenya whereas September is the peak of the wet season. The choice of the two months was to optimize the ecological effects brought about by the two seasons (wet and dry).

**Laboratory analysis**

Microscopic identification and enumeration was conducted a week after sample collection. Firstly, a standard (concentrated) working volume of 50 ml across the collected batch of samples was obtained by incorporating sedimentation and further filtration using a small-modified sieve of 20 µm. Afterwards, 1 ml of the standardized sample was transferred to a Sedgewick rafter cell. One hundred small squares in the Sedgewick rafter cell (0.1 ml in duplicate) were taxonomically identified to genera level (Niklas et al., 1978; Tomas, 1997; Cronberg and Annadotter, 2006), and counted under an inverted microscope (Euromex-Oxion inverso, 200x). Confirmatory iden-

tification for smaller species (<20 µm) was carried out using an ordinary microscope slide under an upright microscope (Zeiss-Primostar, 1000x).

**Statistical analysis**

The data obtained from laboratory analysis was keyed in and processed on an excel spreadsheet. Normality of the data was tested using the Shapiro-Wilk test in R statistical software (version 4.0.2). Indices of diversity such as genera richness, abundance, diversity and relative abundance across the different

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![Map of Kenya showing Kurawa, Kemu and Tana saltworks. Sampling was conducted in Kurawa and Tana saltworks. Adapted from Google Earth. Accessed on 01/07/2022.](image-url)
sampled ponds and seasons were calculated from this data using the formulas i, ii and iii listed below according to Kiteresi et al. (2011). A regression analysis was carried out between the indices of diversity calculated and salinity to establish any form of relationship. All regression analyses were considered significant at \( p<0.05 \).

i) Abundance (\( N \)) = \( A \cdot 1000 \cdot C / V \cdot F \cdot L \)

Where:
- \( N \): Number of plankton cells per liter of the original water
- \( A \): Total number of plankton counted
- \( C \): Volume of final concentrate of the sample in ml
- \( V \): Volume of a field in mm\(^3\)
- \( F \): Number of fields counted

ii) Shannon-Wiener Index (\( H \)) = \( \sum n_i/N \log_2 n_i/N \)

Where:
- \( n_i \): number of individuals of the \( i^{th} \) species
- \( N \): total number of individuals

iii) Pielou evenness index (\( E \)) = \( H/\ln S \)

Where:
- \( H \): Shannon Wiener’s species diversity index
- \( S \): Species richness (number of species)

### Results

#### Water quality parameters

Five water quality parameters were measured in each of the sampled ponds; namely salinity, pH, temperature, conductivity and total dissolved solids (Table 1). Salinity ranged from 37.95 to 197.55 ppt. Temperature ranged from 26.55 to 36.55 °C, whereas pH ranged from 6.43 to 11.87. Conductivity ranged from 26,172.00 to 267,574.00 S/m while TDS ranged from 12,385.50 to 142,362.75 mg/l. Generally, higher salinity ponds were much warmer than the lower salinity ones.

#### Phytoplankton distribution

The present study identified 76 phytoplankton genera distributed among five classes, namely Dinophyceae (13), Bacillariophyceae (38), Cyanophyceae (13), Chlorophyceae (10) and Euglenophyceae (2) (Table 2). The five major genera of Cyanophyceae were *Synechococcus*, *Oscillatoria*, *Lyngbya*, *Anabaena* and *Spirulina*. For Dinophyceae, they were *Gymnodinium*, *Akashiwo*, *Prorocentrum*, *Scrippsiella* and *Dinophysis*. For Chlorophyceae, the major genera were *Dunaliella*, *Tetraselmis*, *Botryococcus*, *Pediastrum* and *Golenkinia*. The major genera of Bacillariophyceae were *Nitzschia*, *Pleurosigma*, *Thalassiosira*, *Navicula* and *Licmophora*. For Euglenophyceae, two genera were identified, namely *Eutreptiella* and *Phacus*. Notably, Cyanophyceae had the highest number of...
genera with the widest salinity tolerance range (Synechococcus, Oscillatoria and Lyngbya) as compared to Bacillariophyceae (Pleurosigma and Navicula), Chlorophyceae (Dunaliella) and Dinophyceae (Gymnodinium).

**Genera abundance**

Genera abundance showed different patterns with increasing salinity in the two sampled saltworks. At Tana salt farm, genera abundance was lowest at salinities ranging from 30-100 ppt, and highest at salinities ranging from 100-200 ppt. Notably, samples collected in March reported higher genera abundance in most of the salinity ranges except at 150-160 ppt where samples collected in September reported significantly higher abundance than those collected in March. With respect to Tana, genera abundance was generally lower in all salinity ranges at Kurawa saltworks. Samples collected in March had higher genera abundance than those collected in September at the salinity range 190-200 ppt (Fig. 2).

**Genera richness**

Genera richness in the sampled salt farms was higher in salinities ranging from 30-100 ppt than in salinities ranging from 100-200 ppt. At Tana salt farm, higher genera richness was observed in most of the salinity ranges except 90-100 ppt where the highest genera richness was observed in March. At Kurawa saltworks, genera richness was generally highest in September for most of the salinity ranges except 90-100 ppt where the highest richness was observed in March (Fig. 3).

**Genera diversity**

Genera diversity in the sampled salt farms was higher in salinities ranging from 30-100 ppt than in salinities ranging from 100-200 ppt. At Tana salt farm, higher genera diversity was observed in most of the salinity ranges during September except at the salinity range 150-160 ppt where higher diversity was observed in March. In Kurawa, higher genera diversity was observed in September in all salinity ranges except 90-100 ppt where the highest diversity was observed in March (Fig. 4).

**Genera evenness**

Genera evenness was highest in salinity ranging from 30-100 ppt except in samples collected in September at Kurawa where genera evenness formed a peak at the salinity range of 150-160 ppt (Fig. 5). Generally, samples collected in September at Tana saltworks produced the highest genera evenness in most of the salinity ranges.
Figure 2. Phytoplankton genera abundance of sampled ponds at different saltworks and seasons. (a) shows abundance in salinities up to 100 whereas (b) shows abundance in salinities above 100. Error bars represent ±standard deviation.

Figure 3. Genera richness of sampled ponds at different saltworks and seasons. Error bars represent ± standard deviation.
except at 150-160 ppt whereas samples collected in September at Kurawa saltworks produced the highest genera evenness in most of the salinity ranges.

**Relative abundance**

**Tana**

At salinity ranging from 30-100 ppt, Dinophyceae and Bacillariophyceae (Fig. 6) dominated phytoplankton communities. Notably, Dinophyceae dominated phytoplankton communities in the salinity range 30-40 ppt in March while in September, Bacillariophyceae were the most dominant. At the salinity range 50-60 ppt, Bacillariophyceae were dominant in March and Dinophyceae were dominant in September. At salinity ranging from 100-200 ppt, Cyanophyceae dominated in both March and September samples.

**Kurawa**

Dinophyceae and Bacillariophyceae dominated phytoplankton communities at salinities ranging from 30-100 ppt (Fig. 7). Notably, Bacillariophyceae dominated in all samples collected in March and September at the salinity range of 30-40 ppt. At the salinity range 50-60 ppt, Bacillariophyceae dominated in September.

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**Figure 4.** Genera diversity of sampled ponds at different saltworks and seasons. Error bars represent ±standard deviation.

**Figure 5.** Genera evenness of sampled ponds at different saltworks and seasons. Error bars represent ±standard deviation.
whereas Dinophyceae dominated in March. At salinities between 100-200 ppt, Cyanophyceae dominated in both March and September samples.

**Relationship between biodiversity indices and salinity**

Genera diversity and richness showed negative relationships with increasing salinity at most of the sampling points and times (Table 3). Genera evenness showed a negative relationship with increasing salinity from 30 to 200 ppt in most of the sampling points except in September at Kurawa salt farm where no significant relationship was observed. Genera abundance on the other hand showed a positive relationship with salinity at Tana in both March and September. At Kurawa, a significant negative relationship between abundance and salinity was observed in September. However, no significant relationship between abundance and salinity was observed at Kurawa salt farm in March.

**Discussion**

The present study identified 76 phytoplankton genera distributed among five classes, namely Dinophyceae, Bacillariophyceae, Cyanophyceae, Chlorophyceae

![Graph showing relative abundance of phytoplankton groups in ponds sampled at different seasons in Tana saltworks.](image6)

**Figure 6.** Relative abundance of phytoplankton groups in ponds sampled at different seasons in Tana saltworks.

![Graph showing relative abundance of phytoplankton groups in ponds sampled at different seasons in Kurawa saltworks.](image7)

**Figure 7.** Relative abundance of phytoplankton groups in ponds sampled at different seasons in Kurawa saltworks.
and Euglenophyceae in the two Kenyan saltworks studied. The number of genera observed in the present study was much higher than in previous studies (Ayadi et al., 2004; Madkour and Gaballah, 2012). Out of the five classes of phytoplankton, Cyanophyceae stood out as the most tolerant class with 3 genera that were present in most of the salinity ranges. High numbers of genera from the class Cyanophyceae (Synechococcus, Oscillatoria and Lyngbya) have also been reported by Nagasathya and Thajuddin (2008) at all sampling points ranging from 48-185 ppt when they studied the diversity of Cyanophyceae in hypersaline environments of saltpans on the southeast coast of India. Further, a review by Oren (2012) noted that some genera in the class Cyanophyceae (Synechococcus, Oscillatoria, Lyngbya, Spirulina, Microcoleus and Synechocystis) could grow at high salinities of up to 200 ppt.

Unlike the other indices of phytoplankton diversity, numerical abundance increased with increasing salinity in all sampling sites and seasons. This was confirmed by the positive coefficient in all regression analyses conducted between numerical abundance and salinity, and this could be attributed to stable (less fluctuating) abiotic conditions associated with hypersaline environments that allow high productivity (Joint et al., 2002). A similar increase in phytoplankton density with salinity was reported by Madkour and Gaballah (2012) when they investigated the phytoplankton assemblage of a solar saltern in Port Fouad, Egypt. Larson and Belovsky (2013) also observed an increase in abundance of Dunaliella spp with increasing salinity when they investigated the influence of salinity and nutrients on phytoplankton communities in microcosm experiments.

**Table 3. Regression analysis between salinity and phytoplankton community structure indices in Kurawa and Tana saltworks.**

| Index        | Sampling site | Coefficient | $R^2$ | $t$ Stat | $P$-value |
|--------------|---------------|-------------|-------|---------|-----------|
| Genera diversity | Tana-March    | -88.36      | 0.75  | -4.91   | <0.05     |
| Genera diversity | Tana-Sept    | -82.32      | 0.93  | 10.45   | <0.05     |
| Genera diversity | Kurawa-March | -91.16      | 0.75  | -4.89   | <0.05     |
| Genera diversity | Kurawa-Sept  | -102.32     | 0.53  | -3.00   | <0.05     |
| Genera abundance | Tana-March   | 0.00        | 0.83  | 6.29    | <0.05     |
| Genera abundance | Tana-Sept    | 0.00        | 0.76  | 5.05    | <0.05     |
| Genera abundance | Kurawa-March | 0.00        | 0.27  | 1.71    | >0.05     |
| Genera abundance | Kurawa-Sept  | -0.04       | 0.64  | -3.79   | <0.05     |
| Genera richness   | Tana-March   | -6.02       | 0.77  | -5.18   | <0.05     |
| Genera richness   | Tana-Sept    | -6.66       | 0.78  | -5.31   | <0.05     |
| Genera richness   | Kurawa-March | -6.14       | 0.82  | -6.08   | <0.05     |
| Genera richness   | Kurawa-Sept  | -4.69       | 0.75  | -4.85   | <0.05     |
| Genera evenness    | Tana-March   | -276.11     | 0.62  | -3.64   | <0.05     |
| Genera evenness    | Tana-Sept    | -251.61     | 0.91  | -8.98   | <0.05     |
| Genera evenness    | Kurawa-March | -251.89     | 0.57  | -3.24   | <0.05     |
| Genera evenness    | Kurawa-Sept  | 102.16      | 0.04  | 0.61    | >0.05     |
It is worth noting that samples collected in Tana saltworks reported higher numerical abundance of phytoplankton genera than samples collected from Kurawa saltworks at all seasons. At Kurawa, a high presence of *Artemia* in the ponds of higher salinity was observed. In fact, *Artemia* were present in some of the samples collected. In Tana saltworks, the density of *Artemia* was minimal and this was attributed to low inoculation by humans due to accessibility challenges, unlike in Kurawa where all ponds were easily accessible. The low numerical abundance of phytoplankton genera in Kurawa saltworks could be associated with the high grazing pressure of *Artemia* in the sampled ponds. The assertion that the presence of high grazing pressure could be responsible for the lower abundance of phytoplankton in Kurawa than in Tana is in agreement with Barnes and Wurtsbaugh (2015), who observed low phytoplankton chlorophyll levels in a microcosm experiment where *Artemia* and other grazers were present, but when the grazers were absent, the phytoplankton chlorophyll levels increased. Karaçoğlu *et al.* (2006) also noted that phytoplankton density changes could be because of predation and grazing pressure through the aquatic food web.

The results of the present have also indicated differences in the phytoplankton dominance among the ponds of different salinities. Ponds of salinities below 100 ppt were dominated by Dinophyceae, Bacillariophyceae and to a lesser extent Euglenophyceae and Chlorophyceae. In salinities above 100 ppt, Bacillariophyceae persisted due to the versatile genera *Pleurosigma* and *Navicula* that were reported even at extreme salinity ranges (Fig. 5 and 6). However, dominance of these two classes was extremely reduced in that their contribution to the phytoplankton communities at the higher salinities (>100 ppt) was negligible. The reduction of dominance of these two classes at salinities above 100 ppt was attributed to intolerance of higher salinities. The finding that Dinophyceae and Bacillariophyceae dominate low salinities (<100 ppt) and the reduction of their dominance at higher salinities (>100 ppt) has also been reported in other studies (Elloumi *et al.*, 2009; Madkour and Gaballah, 2012).

Changing dominance between Bacillariophyceae and Dinophyceae in different seasons was also observed, which Ayedi *et al.* (2004) referred to as a negative correlation between Bacillariophyceae and Dinophyceae dominance. In the marine environment, the shift between Bacillariophyceae and Dinophyceae has been associated with seasonal variations. Hilaluddin *et al.* (2020) observed that Bacillariophyceae contributed the highest percentage (66.0 % to 98.9 %) of the phytoplankton community in most parts of the year under normal conditions, but their contribution declined (43 %) after the wet season with Dinophyceae contributing the highest percentage. While the results from the present study demonstrated the shift between Bacillariophyceae and Dinophyceae in different seasons, a clear association of season with the dominance of either group was not observed.

Cyanophyceae was the most dominant group in ponds of salinities above 70 ppt accounting for >90 %, and to a lesser extent Chlorophyceae, Dinophyceae and Bacillariophyceae. Three genera (*Synechococcus*, *Oscillatoria* and *Lyngbya*) were identified that were exclusively responsible for the dominance of Cyanophyceae. The observed dominance of Cyanophyceae in the present study is not surprising. Das Sarma and Arora (2002) noted that Cyanophyceae dominate the plankton biomass in many hypersaline lakes and form microbial mats. The ability of Cyanophyceae to dominate hypersaline environments has been associated with the accumulation of compatible solutes in their cells to counter high osmotic pressure. For instance, the unicellular species of Cyanophyceae (*Aphanotoece halophytic*) has been observed to accumulate high glycerin in their cells and can tolerate salinity up to 124 ppt (Nagasathy and Thajuddin, 2008).

In terms of the effect of season on biodiversity indices, generally higher genera richness, evenness and diversity were reported in samples collected in September than in March. September coincides with the wet season (southeast monsoon) which is characterized by high organic and inorganic nutrient input into the oceans from the land, whereas March coincides with the dry season (northeast monsoon) which is characterized by limited nutrient input from land. According to Affan *et al.* (2005), phytoplankton abundance and taxonomic diversity depends on supply of nutrients in natural waters, and an increase in phytoplankton diversity with increase in nutrient concentrations in water has been observed. The seasonal differences in organic inputs could be responsible for the differences in phytoplankton diversities and abundances observed between the two seasons during which sampling was carried out.

**Conclusions**

The findings of the study have revealed the presence of highly diverse phytoplankton communities in the tropical saltworks of Kenya. Genera richness was highest
in the low salinity ponds and decreased sharply with increasing salinity gradient. Conversely, the genera abundance increased with increasing salinity forming a peak at higher salinity. Generally, samples obtained in September from most of the sampled ponds reported higher genera richness, evenness and diversity. Ponds of salinity ranging from 30-100 ppt were dominated by Dinophyceae and Bacillariophyceae that showed a shifting dominance. However, it was not clear what the effect of season was on the shifting dominance observed, probably because sampling was restricted to only two months of the year. A follow-up study with year-round sampling is therefore recommended.

In salinities above 100 ppt, Cyanophyceae were the most dominant algae. From the result there was a clear indication that salinity influenced the occurrence and distribution of phytoplankton along the increasing salinity gradient. It is also important to note that genera abundance was much higher in the Tana as compared to the Kurawa saltworks and it is suggested that this was associated with low grazing pressure by Artemia that were present at different densities at the two sites. Lastly, the present study only focused on phytoplankton communities which sit at the base of the food chain powering the biological system in saltwork ponds. For a better understanding of the microorganisms that thrive in the Kenyan saltworks, similar studies investigating the assemblage of other organisms such as zooplankton, halophilic bacteria and macrobenthic fauna are encouraged.

Acknowledgements
The authors would wish to thank Kurawa Ltd for permission to collect samples from their ponds and Kenya Marine and Fisheries Research Institute for the staff and equipment availed during sampling and analysis of the samples collected. The Western Indian Ocean Marine Science Association (WIOUMSA) is acknowledged for sponsoring the study through the ‘Optimizing Artemia Production Technology for Sustainable Aquaculture Development (APTSAD)’ project (MASMA/OP/2020/02).

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