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KENYA Aquatica

A Scientific Journal of Kenya Marine and Fisheries Research Institute

KMFRI

KENYA Aquatica

A Scientific Journal of
Kenya Marine and Fisheries Research Institute

Conference Proceedings of the Kenya Coastal Development Project (KCDP)

Editorial

The current issue of the Kenya Aquatica comprises some papers presented during the Kenya Coastal Development (KCDP) Conference held at the Voi Lodge, Taita Taveta County, from 21st to 23rd March, 2016. This is the initial series of papers submitted by the key authors. It is hoped that the next issue of the Journal, more papers will be compiled from the KCDP Conference proceedings. The Journal Editorial Board has compiled and published these papers with support from KMFRI.

This initial Volume contains papers on themes covering water resources and environmental sustainability; engineering technology and innovation; agriculture, aquaculture and biotechnology; marine resources and fisheries.

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About Kenya Aquatica

Kenya Aquatica is the Scientific Journal of the Kenya Marine and Fisheries Research Institute (KMFRI). The Aim of the Journal is to provide an avenue for KMFRI researchers and partners to disseminate knowledge generated from research conducted in the aquatic environment of Kenya and resources therein and adjacent to it. This is in line with KMFRI's mandate to undertake research in marine and freshwater fisheries, aquaculture, environmental and ecological studies, and marine research including chemical and physical oceanography.

Manuscripts may be submitted to the Chief Editor through Director@kmfri.co.ke

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Contents

Access to Water in Kenya's Coast Region: A Challenge to Community Development and Poverty Alleviation in Lamu County

David O. Ignatius, Farida A. Hassan, George N. Morara, Melckzedeck K. Osore, Benedict Mutua and Nzula K. Kitaka

1 - 7

Biochemical and Sensory Characteristics of the Smoked African Catfish (Clarias Gariepinus) under Different Storage Conditions in the Coastal Region of Kenya

Maurice O. Obiero, Peter M. Oduor-Odote, Raymond Ruwa and M. Omega

8 - 12

Evaluation of UV-Resistance of Epibiotic Bacteria Co-existing with the Kenyan marine *Lyngbya majuscule*

Constance Nyiro, Thomas Dzeha, Joseph Mwafaida and David Mburu

13 - 16

Identification and characterization of Kenyan Marine Microalgae Strains towards Biofuel Production

Kathleen Musungu, Allan Mgutu, Charles Matoka and Josiah Odalo

17 - 22

Long Line Seaweed Farming as an alternative to other Commonly used Methods in Kenyan Coast

Nyamora J. Moraa, Nyakeya Kobingi, Esther Wairimu, Gladys Mwihaki and Judith Muya

23 - 28

Review of Coral Classification Status: A Case Study of Kiunga Marine National Reserve, North Coast Kenya

Josphat Nguu, David Obura, Michael Murunga and Bernerd Fulanda

29 - 37

Influence of salt works' hyper-saline waste-brine on distribution of mangrove crabs (Decapoda) within the Gongoni-Kurawa intertidal area, Kenya

Sonia K. Mumbah, Julius O. Manyala, James Njiru

38 - 47

Kenya Aquatica Journal - Volume 4, Issue 01 (1 - 7)

Access to Water in Kenya's Coast Region:
A Challenge to Community Development and Poverty Alleviation in Lamu County

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Abstract

Water is considered a basic commodity and essential for life - living on planet earth is dependent on it. However, access to water has been and will continue to be a dilemma for a majority of the residents at the coast of Kenya. A close look at the window on "Coastal Resources and People" reveals that water is a key resource but despite its immense importance, many people especially in the rural areas and among the Vulnerable and Marginalized Groups (VMG's) do not have adequate access to potable, reliable and convenient sources of water. Lamu, currently considered among water scarce counties in Kenya as per the Lamu County Integrated Development Plan (http://lamu.go.ke/wp-content/uploads/2016/03/LAMU_CIDP-Revised.June_2014-1.pdf), faces serious challenge of provision of potable water to its residents. With the influx of people from other parts of the country as a result of the implementation of the Kenya Vision 2030 flagship project - Lamu Port Southern Sudan Ethiopia Transport (LAPSSET) Corridor, the current water stresses is only expected to worsen. The Lamu County Government plans to address the ever-increasing demand for access to water but resources to actualise these plans are yet to be consolidated. To complement this situation, Kenya Coastal Development Project (KCDP), a World Bank funded project is working with local communities in Lamu in the development and implementation of community-led water projects targeting to increasing access to water at the household level.

This paper will focus on community-based approaches to understand the water-web in Lamu East Sub-County to provide an assessment of opportunities, challenges and sustainability implications. Primary data is used on surveys, Social Assessment (SA),Vulnerable and Marginalised Group Plan (VMGP), observations and recommendations from the Lamu CIDP. It further proposes that direct usage of saline water, seawater or brackish water, for sanitation purposes could alleviate the freshwater shortage.

Key words: Water-web, challenge, sustainable development, community driven demand.

INTRODUCTION

The 6th and 7th Goals of the Sustainable Development Goals (SDGs) and Millennium Development Goals (MDGs) respectively focused in part on water scarcity, with a target of halving the number of people around the world without sustainable access to safe drinking water and basic sanitation services. Today, 91% of the world's population uses an improved drinking water source, compared to 76% in 1990, but water scarcity still affects more than 40% of people, across every continent (UNDP, 2006).

Water is crucial in several other sectors apart from the portable water and sanitation. As highlighted by the United Nation Millennium Project (2004). The United Nation's General Assembly through Resolution 64/292 explicitly recognises the human right to water and sanitation and acknowledged that clean drinking water and sanitation are essential for the realization of all human rights. This is fortified by the Kenyan Constitution promulgated in 2010 and further strengthened by Kenya Vision 2030 and the Ministry of Environment Water and Natural Resources Strategic Plan 2013 – 2017. Under Article 28, of the Kenya Constitution 2010, every person has inherent right to have the dignity respected and upheld. The human right to good quality water is indispensable for leading a life in human dignity. The Kenya Constitution 2010, states that "every person has inherent dignity and right to have the dignity respected and upheld". The human right to good quality water is indispensable for leading a life in human dignity.

Kenya has been classified as a water scarce country by World Health Organisation (WHO, 2003). Only 48% of the country's rural population has access to an improved drinking water source according to Kenya National Bureau of Statistics (KNBS) and Society for International Development. Fig. Shows the percent availability of water in household of Lamu County.

Improved sources of water comprise protected spring, protected well, borehole, piped into dwelling, piped and rain water collection while unimproved sources include pond, dam, lake, stream/river, unprotected spring, unprotected well, djabia, water vendor and others

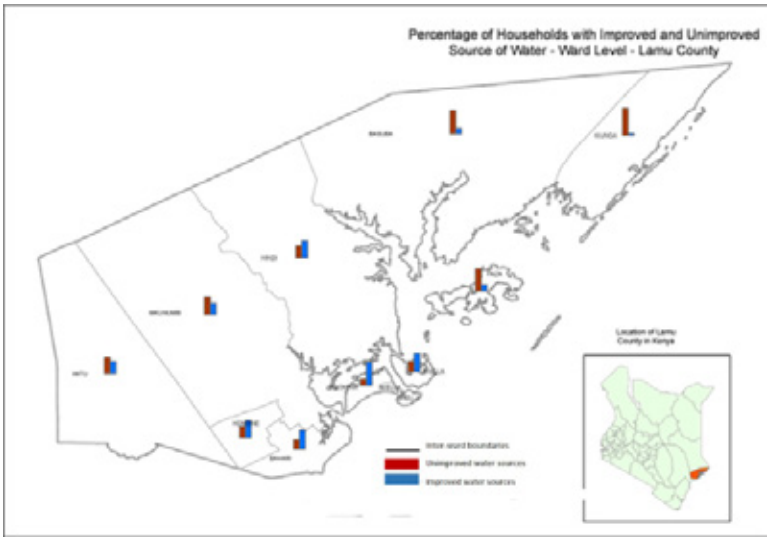

Fig. 1: Lamu County summary of households with improved and unimproved source of water at the Ward level based on Kenya National Bureau of Statistics. Improved sources of water comprise protected spring, protected well, borehole, piped into dwelling, piped and rain water collection while unimproved sources include pond, dam, lake, stream/river, unprotected spring, unprotected well, djabia, water vendor and others.

The time spent in pursuit of water collection often prevents people, particularly women, from concentrating on income generating activities, or in the case of school going children, leads to poor school attendance and performance. Fig. 2 shows a typical scene at a water collection point. Due to the water scarcity in the rural areas, waterborne diseases are not uncommon.

Lamu County has land surface area of 6,300 km^2 composed of 5,500 km^2 of arable land 800 km^2 of non-arable land,130 km^2 of coastline and 308 km^2 under water mass. Lamu West sits on land surface area of 3,970 km^2 hence taking 63.3% of total land, leaving Lamu East with 36.7%. Kiunga division in Lamu East occupies 96.6% of Lamu East land surface area. The bulk of arable land is in Lamu West while Lamu East takes the bulk of water mass.

The County covers a strip of north-eastern coastal mainland and the Lamu archipelago, which consists of around 65 islands, which extend about one hundred kilometers south from the Somalia border. The most well-known of the islands is Lamu Island, and is termed as "the oldest and best-preserved Swahili settlement in East Africa" in the CIDP 2013 – 2017 but neighbouring islands also have numerous archaeological remnants of history dating as far back as the 14th Century (https://www.pinterest.com/pin/558657528756715021/).

It has been suggested that most of this settlements may have collapsed in parts of the archipelago and its hinterland due to lack of access to fresh water. However Lamu Island has continued to thrive as a result of its reliable sand dune recharged aquifers. Rain Water Harvesting (RWH) has however been embraced in the other islands with the construction of the underground RWH storage tanks, locally known as djabia. The current water is greatly supplemented by saline or brackish water boreholes.

In Lamu County, 53% of residents use improved sources of water, while the rest relying on unimproved sources. There is no significant gender differential in the use of improved sources of water, with 53% of male headed households and 54% in female headed households using improved water sources respectively. Lamu West constituency has the highest share of residents using improved sources of water at 61%. That is three times Lamu East constituency, which has the lowest share using improved sources of water. Lamu West constituency is 8 percentage points above the county average (Fig. 1).

On the other hand total of 57% of residents in Lamu County use improved sanitation, while the rest use unimproved sanitation. Improved sanitation is higher in female headed households at 61% as compared with male headed households at 55% (KNBS and SID, 2013). Lamu East constituency has the highest share of residents using improved sanitation at 72%. That is 19 percentage points above Lamu West constituency, which has the lowest share using improved sanitation. Lamu East constituency is 15 percentage points above the county average. Faza Ward has the highest share of residents using improved sanitation at 78%. This is almost 51 percentage points above Witu Ward, which has the lowest share using improved sanitation. Faza Ward is 21 percentage points above the county average.

Although the link between water and poverty may be easy to grasp, the issue of how to safeguard our water so that the communities can gain access to the resource necessary for consumption and production is still complex and needs close attention.

This paper seeks to address the following:

- Water and poverty nexus in Lamu County.
- Compare the cost effectiveness of KCDP approved projects, based on Rain Water Harvesting (RWH) techniques in Lamu County as prioritized via Community Driven Development (CDD) approach.
- TPresent the case of water needs for the Vulnerable and Marginalized Groups (VMG).
- Highlight alternative ways of addressing water management to meet the current water demand based on the cultural practices by the indigenous communities of supplementation utilization of saline or brackish water.

METHODS

Based on the current KNBS statistics and Lamu County water department, 28 villages were studied to identify the water and poverty nexus in Lamu archipelago and its hinterland. 16 villages in Faza, Kiunga and Basuba Wards were considered. These villages depended on RWH using the underground water tanks, locally known as djabia as their major source of freshwater. The number

Fig. 2: Women and girls queuing for water from an opened underground water tank, djabia in October 2013 – the month when water is usually scarce. The queues are long that may lead to conflicts and they take more than WHO standard of not more than 30 minutes to draw water from one point even though they are not located Dfar from the households.

of current available djabia's, and their storage capacity and the total village populations were identified to determine the prevailing freshwater deficit based on the WHO standard of 50 litres of water per individual per day.

On the other hand, the 12 villages inhabited by the Aweer (Boni) tribe considered to be the poorest were engaged. They are among the Vulnerable and Marginalized Group (VMG) as defined in article 260 of the Kenya Constitution and includes groups covered by the World Bank's OP 4.10. article 56 which mandates the State to undertake affirmative action programmes to fast track the integration of minority and marginalized communities into the mainstream social and economic life of Kenya; article 204 (l) which establishes the Equalization Fund for fast tracking development of basic services such as the provision of water to bring them to per with other areas of Kenya; article 174 (e) which mandates the county government to protect and promote the rights of minorities and marginalized communities.

The Social Assessment (SA) in which a sample of VMG villages participated actively in formulating action plans was followed by validation workshop in which representatives of VMGs from the sample villages had the opportunity to discuss the SA findings, make amendments and give their approval for the findings and prepare a Vulnerable and Marginalised Groups Plan (VMGP). Each village being represented by a total of 8 persons, undertook the identification, prioritization and budgeting for their preferred and culturally appropriate social economic-livelihoods and micro-enterprise project to achieve sustainable development.

Built on VMGs representative prioritization and ranking of their major projects namely water, public sanitation, education, health, infrastructure support, agriculture, micro-enterprise, tourism and fisheries sub-sectors. The village's need of water, the community proposed source of water and distance to water points were provided as summarised in the VMGP.

On the other frontier, based on the three Rain Water Harvesting (RWH) projects approved through KCDP grants award on the Community Service (CS) window to the Community Based Organisations (CBOs). The cost effectiveness of the traditional underground tank, convectional roof top catchment and novice use of plastic tanks was evaluated. This is through the Community Driven Development (CDD) projects awarded to three community based organisations in Lamu East Sub-county villages of Siyu, Ndau Island and Tchundwa to two CBOs namely: Girl Child Protection, youth and disabled community groups respectively.

RESULTS

The results show the achievement of the MDGs, and further explore challenges to sustainable implementation of RWH and proposes and some interventions which the government and other stakeholders could implement to overcome them.Fig. 3shows graphic presentation of water deficit situation in various villages of Lamu in along the scale of the MDG target for 2015.

All of the 16 villages water demand is a priority apart from Faza/Rasini which is the most developed village and is the proposed beneficiary of Iranian funded water project that plans to supply borehole water through from the mainland in Vumbe to Faza Island though undersea water piping system. Kizitingini which is the most densely populated (25% of the threshold) and Kuinga's Shanga Village and Basuba are the hardest hit as water does not achieving the 7th MDG. None of the villages has met the MDGs threshold of more than 50% access to portable water.

The water need as prioritized by the Aweer (Boni) tribe show that apart from Mansghuda and Malkamansa villages the rest of the village's prioritized water as zenith to their sustainable development in comparison to other social-economic projects. The ranking was inversely proportional to the distance to water point, while it was directly proportional to the availability of shallow wells that are functioning. Fig. 4 Shows the water deficit situation of the villages occupied by the Boni VMG.

Fig. 3: Water-web on the percentage deficit of water supply based on the Millennium Development Goals (MDGs) target of supply of water to 50% of the population by 2015 is far from being achieved in the case of Lamu Archipelago and its hinterland. They highlight water demand for some of the major villages based on the current standards by World Health Organisation (WHO) that recommend minimum of fifty litres of water per individual per day and the water source has to be within 1 kilometres of the home and collection time should not exceed 30 minutes.

It is worth noting that most of the shallow wells dry during drought periods and through 9 out of 12 villages were advised to sink boreholes, the high salinity content of such ventures are a major risk. The 3 of the villages opted for traditional RWH using djabias. This has however avoided during the appraisal of more than 20 CDD projects as had been proposed by CBOs in Lamu archipelago. These decisions can be validated by lessons learned by KCDP was based on several factors that stand out during the approved water project in Lamu County.

Three CBOs namely Siyu Girl Child Protection Youth Group, Muungano wa Walemavu and Al-Fattah Self Help Group had their RWH projects approved,. Their community driven development projects to improve access to domestic water for Siyu Village to benefit the girl child, roof water harvesting project for the physically challenged persons and a convectional djabia rehabilitation in Ndau Island by a youth self-help group. The rain water structures are highlighted in Fig. 5 showing in each case the situation at the project site during inception and after completion ().

From the active projects the following projects were identified and their capacity to hold water, catchment surface area, cost, drawing point, benefiting community members among others factors that enable the determination of the best practice determined. The greater the values that directly account to

Fig. 4: Water-web based on the current Vulnerable and Marginalised Group Plan (VMGP) after Social Assessment for the Aweer (Boni) tribe in Basuba Ward. Ranking of villages based on their water needs, other factors such as prioritized rank among other projects, distance to water points, shallow wells available and functional shallow wells are considered.

the improved RWH, the better the method (Fig. 6).

The RHW method can be categorised into three, namely the Traditional djabia, Convectional/Improved djabia and Modern protected plastic water tank.

Lastly, informed about the Lamu archipelago culture and studies over the current epoch, a group of researchers from Hong Kong, The Netherlands and South-Africa developed, tested and applied a novel approach to water management in coastal cities, where saline water is used as secondary quality water. This is based on the fact that desalinisation is still a very expensive process that needs a lot of energy, plus the brine waste of high salinity from the process is still hard to dispose. Neophyte approach addressing traditional issues such as the need for a dual water system, requirement for use of non-corrosive materials, issues with H_2S formation, impact of increased salinity on biological wastewater treatment, reduced reuse options due to saline effluent, and lack of proper cost-benefit analysis of different options, in a holistic fashion and introduces novel technological interventions and developments at various parts of the urban water infrastructure system. Direct use of saline water replaces a substantial part of freshwater usage, exploits sewerage as a bioreactor and introduces new Sulphate reduction, Autotrophic denitrification and Nitrification Integrated (SANI) process technology for treatment of sulphate-rich wastewaters. This concept has evolved over the last 50 years in Hong Kong and has recently matured to the degree that can be applied at full scale for sustainable development in proposed the LAPSSET resort cities.

DISCUSSION

The delicate link between access to water and poverty reduction was weakened when the decade of water declaration became operational into MDGs. Where halving the world's poor population become Goal No. 1 while the issue of securing access to safe drinking water only became a target under Goal No. 7, on 'Ensuring Environmental Sustainability'. However, the goal of halving the number of people without access to clean water is turning to be one of the most cited and well known of the MDGs. It has turned out to be one of the most difficult to achieve as in the case of Lamu County.

The county population as projected in Lamu stands at 124,092 persons (https://www.knbs.or.ke/download/statistical-abstract-2015/), with 64,827 male and 59,265 female and more than 70% live in Lamu West. The envisaged opportunities from the LAPSSET project is expected to attract huge influx of immigrant population estimated to be over 1.0 Million. This will certainly overstretch the county's social services necessitating commensurate development planning for adequate service provision. This will bring about dimensional shift in the county's sustainable development needs.

Water is an important factor in settled communities, and the evolution of public water supply systems will be tied directly to the growth of Lamu County. The

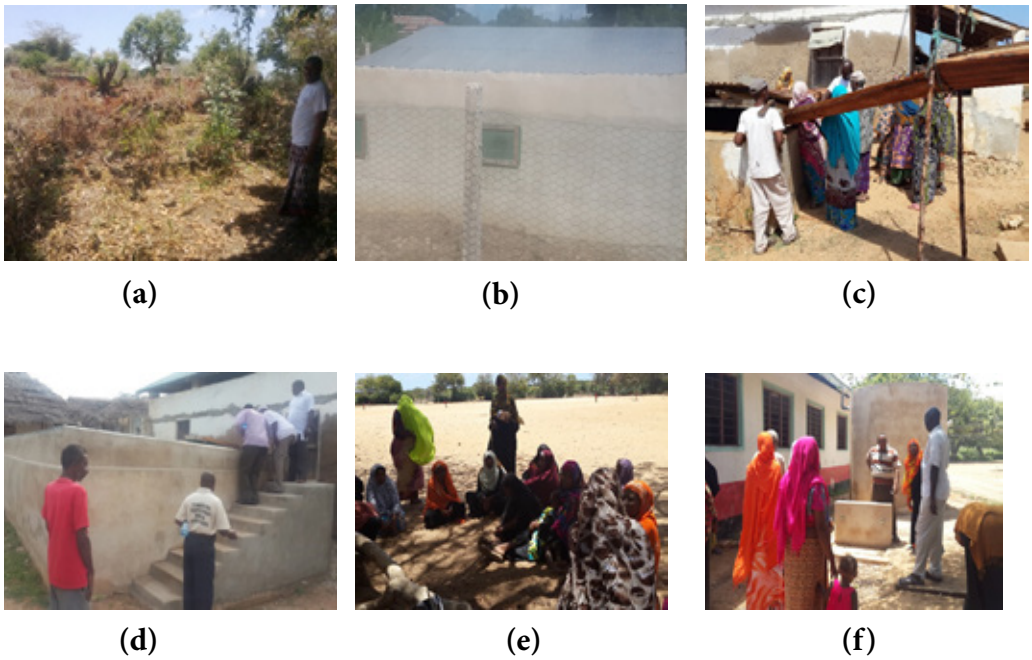

Fig. 5: Plates on transformative status of the water of the 3 funded Rain Water Harvesting (RWH) projects funded by Kenya Coastal Development Project (KCDP) in Lamu Archipelago. Plates (A – B), (C – D) and (E – F) highlight the former and current state of project site for Girl Child Protection Youth Group, Al-Fattah Self-Help Group and Muungano wa Walemavu Self Help Group respectively.

county is among the regions that suffer from historical marginalization. The low development achieved is also concentrated in Lamu West Sub-County while Lamu East Sub-County continues to suffer massive under development to date as came out in the Lamu CIDP.

To call water the basis of life doesn't give it enough credit, yet we often treat water as an afterthought, until it's gone in to the ground for us to begin exploiting it. Already, 1.2 billion people, or nearly a sixth of the world's population, live in areas afflicted by water scarcity, and that Fig. could grow to 1.8 billion by 2025 (http://www.un.org/waterforlifedecade/scarcity.shtml). Globally, the rate of water withdrawal water diverted from an existing surface or underground source increased at more than twice the rate of population growth over the past century. Climate change could intensify desertification in already dry parts of the planet.

The world is projected to hold 9 billion people or more by 2050 and they'll all be thirsty. So in now and beyond, the challenge of water scarcity will only grow, which could lead to global instability. But it doesn't have to be that way. Efficiency can stretch existing supplies by ensuring that overall water use fall even as the population grows. The truth is that we can do anything with water – except go on without it. Water produced by the plant is tasted for quality before it is sent out for consumption at a premier cost. But for the less privileged RWH still remains the principle source of freshwater freely available from nature.

Water and sanitation facilities and services must be available and affordable for everyone, even the poorest. The costs for water and sanitation services should not exceed 5 per cent of a household's income, meaning services must not affect peoples' capacity to acquire other essential goods and services, including food, housing, health services and education.

Water is inseparable with sustainable human economic and social development. One of the eight MDGs and eighteen targets, target 10, is on water supply services. Water underpins virtually all the MDGs. Water has many quite fundamental and quite different facets and functions in human societies. Therefore, the assessment of the role of quality water as the concept of water as a human right are not trivial. The importance of water and sanitation as drivers for health, quality life, food security and as a pillar for economic development is unique. Besides being fundamental to many economic sectors, water is also a key to meeting many of the basic needs that are in turn instrumental in poverty reduction.

Prior to 2002, the government of Kenya had focused on nationalizing water supply through a centralized body and large-scale water projects, including the promotion of the goal of universal water supply by the year 2000. However, there was a growing realization that the government lacked resources and capacity to attain such goals, and that alternative solutions would need to be found. With the introduction of Kenya's Water Act of 2002, the national government provided more freedom and autonomy to allow other government and non- government bodies to supply water by providing a more conducive environment to facilitate a decentralization of water supply. As a result, new water sector institutions have been created, and new ideas for water supply have come about. One of the ideas gaining attention with this new system has been RWH, which may previously have been treated as an afterthought or lacked applicable knowledge. With this growing awareness and interest, there is increasing possibility for RWH to be incorporated in water supply strategies. Halving the proportion of people without sustainable access to safe drinking water and basic sanitation, is one of the targets of the 7th MDGs (Onda et. al. 2012). RWH, which provides water directly to households enables a number of small-scale productive activities, and has the potential to supply water even in rural and peri-urban areas that conventional technologies cannot supply. As part of the effort to achieve the MDGs, the government has committed itself to provide financial assistance to poor households for the capital cost of rainwater storage tanks and related works in the rural areas. Despite this financial assistance, the legal status of RWH remains unclear and RWH is in fact illegal by strict application of the water legislations. Beyond the cost of installation, maintenance and proper use of the RWH system to ensure its sustainability, there is risk of waterborne diseases.

Lamu is proposed to have "Collaboration City" located at Mokowe with the core facilities such as water sports, country club, fishermen's wharf, cultural center, convention center and amusement center (as part of the Kenya's Vision 2030). The Satellite facilities are eco-tour points include marine and land sports, fishing, market, surfing, nature safaris and archaeological sites. The eco-villages are proposed at Kipini, Bawaya, Manda Island, Pate Island, and Kiwayuu Island all this will have a lasting stress in the available freshwater resources or its availability will be unadorned minimum. The main sources of water include rainfall, surface water, groundwater, and desalination of sea water. Ground water sources are the major water sources for most of the water supplies in Lamu, with most areas in the County having saline groundwater. Surface water sources include the lakes, pans, dams, seasonal rivers and the sea.

Water in the county is managed by various institutions. Lamu Water and Sewerage Company (LAWASCO) controls the distribution right to Mokowe and Lamu water systems. Lake Kenyatta Water Association supplies water to Mpeketoni area, Witu and Hindi Water User Associations manage water supplies in Witu and Hindi areas and are community based schemes. Other public water sources such as dams and djabias are managed by community committees. Rain harvesting structures are used to collect water for domestic use. Desalination of sea water is mainly done by hotels and private individuals since the process is costly and the average distance of household to access clean water is 5 kilometres.

Of all services for sustainable development, provision of potable water is perhaps the most vital. People depend on water for drinking, cooking, washing, carrying away wastes, for livestock and other domestic needs. Water supply systems must also meet requirements for public, commercial, and industrial activities. In all cases, the water must fulfil both quality and quantity requirements. This is the greatest dilemma that sustainable development in Lamu County does face. Water supply system, infrastructure for the collection, transmission, treatment, storage, and distribution of water for homes, commercial establishments, industry, livestock and irrigation, as well as for such public needs as fire fighting and street flushing is a necessity.

Water is present in abundant quantities on and under the Earth's surface, but less than 1 percent of it is liquid fresh water. Most of Earth's water is in the oceans or frozen in polar ice caps and glaciers. Ocean water contains about 35 grams per litre of dissolved minerals or salts, making it unfit for drinking and for most industrial or agricultural uses. There is ample fresh water—water containing less than 3 grams of salts per litre,—to satisfy all human needs. It is not always available, though, at the times and places it is needed, and it is not uniformly distributed over the Earth. In many locations as is the case of Lamu the availability of good-quality water will be further reduced because of urban development, industrial growth, and environmental pollution.

Uncontrolled environmental degradation and effects of climate change negatively impact on the socio-economic sustainable development of the county.

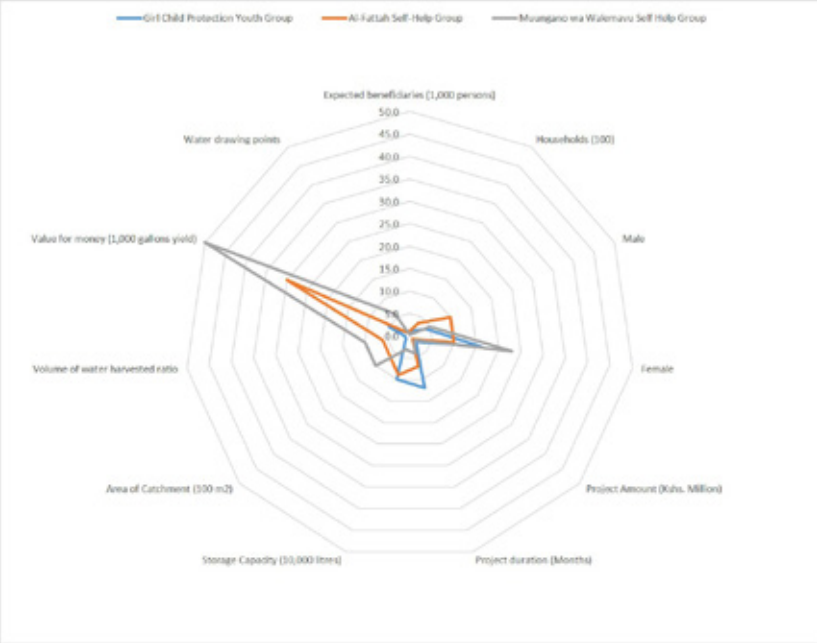

Fig. 6: Water-web comparing three methods namely i) Traditional underground rainwater storage tank. ii) Convectional roof catchment to a raised storage tank and iii) Roof with a protected plastic water tank, of Rain Water Harvesting (RWH) in Siyu, Ndau and Tchundwa villages respectively as funded by Kenya Coastal Development Project (KCDP).

Human activity is the major contributor to environmental degradation in Lamu County. These activities include deforestation through illegal logging, charcoal burning, forest clearing for agricultural activities, overstocking and subsequent overgrazing, illegal quarrying and water pollution through waste disposal. The depletion of mangrove forest reduces reproduction of marine life, deforestation and overgrazing leads to desertification and reduce rainfall and water sources, water pollution leads to water borne diseases while climate change has increased the frequency of high tide flooding.

KCDP supports projects geared towards maximizing the social benefits and promoting services and actions that enhance social-wellbeing in the six coastal counties. The pivotal recipients are vulnerable groups, women, youth and self-help groups pulling together with a common interest to provide solutions to their teething problems but are in need of financial boost to address their recurrent problems. During the first round of the project more than twenty water projects from Lamu were put on hold awaiting scientific sound practices. This action is clearly justified from by the current complete RWH projects. This has resulted to the county receiving the lowest of approved projects requested by the CBOs. This would have otherwise gone a long way in addressing a clear demand of fresh water, because the region is located in the Arid and Semi-Arid Lands (ASAL).

The Aweer are a remnant hunter-gatherer group living along the Kenyan coast in the North-Eastern parts of Lamu County on the mainland. The community lives in a total of 12 villages in the forested areas within Witu and Boni forests with a population of about 8,000 persons (KNBS, 2009, Kenya Population and Household Census, Page 397). The gazettement of all the forest by the government has become a source of conflict. To ensure social, economic and cultural benefit to the VMGs poverty must be lifted and access to water is aimed at bringing them at per with other communities in the Coast and Kenya at large. The applied interventions must be culturally appropriate and aim at preserving and promoting cultural practices and income generating. This can be achieved by ensuring the free, prior and informed consultations to enable their involvement and participation at every step of VMGP implementation.

The VMGP provide a clear road map on the needs and aspirations of the Aweer (Boni) tribe. They present measures and actions to be taken in order to strengthen and enhance the social, livelihoods and economic visions so as to uplift their quality of life. It further provide measure for mitigating and perceived adverse effects that may result from the implementation of KCDP. It also provide a clear mechanisms for sustainably engaging the local communities to participate and benefit from project's interventions and investments. Provision of water if implemented as provided in the VMGP will be capable of fast tracking the integration and inclusion into the mainstream social-economic sustainable development of Lamu as envisaged by article 56 of the Constitution of Kenya, 2010.

Safe domestic water is a basic necessity for good health. In addition it is particularly significant for women and children, usually girls in rural areas, who bear the primary responsibility for carrying water, often from long distances. The Aweer rely on borehole /open well and spring/ river/ponds which frequently dry up during dry seasons. However, scarcity of water among the tribe is a barrier to irrigated agriculture which has high potential in the areas occupied in KCDP project area.

As a result of combined effects of population growth, rapid urbanization, densification of urban area and climate change, people are increasingly experiencing freshwater shortages in Lamu and the situation is expected to be only worsened in the near future. Traditional mitigation measures, like introduction of water saving devices or RWH, are often insufficient in solving the problem. Therefore, novel alternative urban water management strategies need to be further explored and developed as an answer to ever increasing water stress. These strategies should preferably be focused on the reduction of the demand for freshwater and introduction of new approaches to urban water management that often includes a paradigm shift.

The main concerns affecting abstraction of groundwater from the Lamu archipelago and its hinterlands current freshwater aquifers include, sea water intrusion, depletion of fresh groundwater resources, over abstraction on water resources by developers and the local communities, reduction of natural vegetation, forest cover and agricultural land due to un-planned developments. This leaves harnessing of rainwater harvesting and use of saline and brackish water as key frontier in addressing the fresh water demand.

Recommendations

The poor continue to have insufficient water as long as the belief persists that there are no alternatives or that the cost are too high. The responsibility to invest in water rests not only with the Ministry of the central government but also with County governments and other stakeholders, but ultimately it is also a responsibility of every household as good quality and fresh water for the poor pays both economically, socially, health wise and environmentally.

Direct saline water, seawater or brackish water, usage for toilet flushing could alleviate the freshwater shortage, but its worldwide application was hindered due to several issues and challenges. The introduction of saline water to the urban water system is relatively simple, safe, sustainable and economically

affordable in urban coastal areas. The novel approach if successful in Lamu archipelago will addresses major concerns and challenges that hindered its wider use in the past and opens window of opportunities for its worldwide application as about 50% of the major cities are have easy access to this ubiquitous marine resource leaving Lamu County as a trailblazer.

Finally, as proposed in the Lamu County Integrated Development Plan (CIDP), the use of ubiquitous amount of saline or brackish water is the way out of the current water quagmire. This has been the culture of all Lamu archipelago natives who have uniquely been able to continue to live without the basic human right of water, affecting their health, safety and survival even though they are among the world's most marginalized and impoverished families. The paper calls for urgency in providing of scientifically backed technology to increasing the magnitude of use of saline water in bathing and sanitation options. This is not only been proven to be cost effective and therapeutic, but also economically viable as in the case of Hong Kong which has practiced it for over 5 decades.

ACKNOWLEDGEMENTS

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REFERENCES

Global Change. (1992). Joint Global Ocean Flux Study, Implementation Plan. JGOFS, Report No. 9. Scientific Committee on Ocean Research International Council of Scientific Unions. International Geosphere-Biosphere Programme: A Study of Global Change (IGBP) and the Scientific Committee on Oceanic Research (SCOR) Both of the International Council of Scientific Unions (ICSU), (9). Retrieved from http://ijgofs.whoi.edu/Publications/Report_Series/JGOFS_9.pdf

Global Change. (1993). Land-Ocean Interactions in the Coastal Zone: Implementation Plan. International Geosphere-Biosphere Programme: A Study of Global Change (IGBP) of the International Council of Scientific Unions (ICSU), (33). Retrieved from http://www.igbp.net/download/18.950c2fa1495db7081e194f/1430900189660/IGBP_report_33-LOICZ.pdf

Lamu County. (2014). First County Integrated Development Plan 2013-2017. Lamu County: Lamu County. Retrieved from http://lamu.go.ke/wp-content/uploads/2016/03/LAMU_CIDP-Revised.June_2014-1.pdf

OHCHR. (2010). The Right to Water. UN Office of the High Commissioner for Human Rights (OHCHR), (35). Retrieved from https://www.ohchr.org/Documents/Publications/FactSheet35en.pdf

Onda, K., Lobuglio, J., & Bartram, J. (2012). Global access to safe water: accounting for water quality and the resulting impact on MDG progress. World Health & Population, 14(3), 32–44.

Rop, R. R. (2010). Gender in Water and Sanitation. Water and Sanitation Program. Retrieved from https://www.wsp.org/sites/wsp.org/files/publications/WSP-gender-water-sanitation.pdf

UN Economic and Social Council. (2003). General Comment No. 15 (2002) The right to water (arts. 11 and 12 of the International Covenant on Economic, Social and Cultural Rights). Agenda, 11, 29.

UN General Assembly. (2010). The human right to water and sanitation. United Nations. Retrieved from https://documents-dds-ny.un.org/doc/UNDOC/GEN/N09/479/35/PDF/N0947935.pdf?OpenElement

United Nations Human Settlements Programme. (2007). Centre on Housing rights and Evictions (COHRE), American Association for the Advancement of Science (AAAS), Swiss Agency for Development and Cooperation (SDC). Manual on the Right to Water and Sanitation. United Nations Human Settlements Programme UN-HABITAT. Retrieved from http://www.unhabitat.org/pmss/listItemDetails.aspx?publicationID=2536

Watkins, K. (2006). Human Development Report 2006-Beyond scarcity: Power, poverty and the global water crisis. Retrieved from https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2294691

WHO. (2015). The Right to Water. 2003. Office of the High Commissioner for Human Rights (OHCHR) Centre on Housing Rights and Evictions (COHRE). Retrieved from http://www2.ohchr.org/english/issues/water/docs/Right_to_Water.pdf.

WSSCC/Gender, & Water Alliance. (2006). For Her It's the Big Issue - Putting Women at the Centre of Water Supply, Sanitation and Hygiene - Evidence Report. Retrieved January 4, 2015, from https://www.wsscc.org/resources-feed/big-issue-putting-women-centre-water-supply-sanitation-hygiene/

Biochemical and Sensory Characteristics of the Smoked African Catfish *(Clarias gariepinus)* under Different Storage Conditions in the Coastal Region of Kenya

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Abstract

Smoking is one of the oldest methods of drying food for purposes of preservation and flavouring. However, technologies are changing fast resulting in various smoking techniques to improve fish quality and shelf life. Different storage conditions have been used and others are still being tested to improve shelf life of smoked fish products. The aim of this study was to determine the changes in biochemical and sensory attributes of smoked African catfish (Clarias gariepinus) under different storage conditions. Samples were stored in open air, sealed polythene paper bags and vacuum sealed polythene paper bags for a period of 30 days. Storage in open air exhibited highest value at day 30 while vacuum package exhibited the least. Protein decreased with time to lowest value of 60.83% in the open air storage at day 30 while those of vacuum packaging exhibited insignificant change with time. Fat content showed no significant linear change with storage time except the one stored under normal packaging. Ash and moisture content exhibited linear change with time on open package samples only.

Organoleptic responses remained constant until day 30 when there was a slight change in responses on taste and overall acceptability. In general, pronounced changes were exhibited on open air while vacuum packaging had the least changes.

Key words: Smoking, Shelf life, Organoleptic, Kilns, Packaging, Quality

INTRODUCTION

One of the oldest methods of drying food for purposes of preservation and addition of flavour is smoking. It is estimated that 25% of the world's fish catch destined for human consumption is dried in some manner, combined with some salting, brining or smoking. The basic food process called drying, combined with effect of salt and smoke particulates results in smoked products (Pigott, 2002). Hot and cold smoking methods are the predominant ones as far as fish smoking is concerned. However, since the beginning of fish smoking, the general procedure has remained unchanged with methods applied depending on opinion. Basically, it depends on which type of wood to use and what temperature and time to do the smoking (Pigott, 2002). This has necessitated the emergence of various smoking technologies (Oyaro et al. 2012) to ensure quality products with improved shelf life in order to satisfy market requirements. However, due to diverse consumer needs, new innovations are still required to further improve the quality of smoked products as well as their shelf life. The African catfish is one of the fishes caught in Lake Kenyatta (oxbow lake of River Tana) in Lamu County coast of Kenya. It is mainly sold as smoked products (tonzi) with a wide distribution in Kenya. However, the quality of the product during storage has been of concern. This follows the understanding that fish is classified as a highly perishable food commodity with shelf life dependent on the initial quality, processing technology and storage conditions. In this study therefore, an improved smoking Kiln was designed with enhanced smoking features for quality products with better characteristics. Biochemical and sensory methods were used in evaluating the characteristics of the smoked products under different storage conditions with the aim of establishing the most appropriate conditions of storage that would improve the shelf life of the smoked fish for marketing purposes both locally and regionally. The packaging types used for storage was open air (as control), normal polythene packaging and vacuum packaging.

MATERIALS AND METHODS

Smoking Protocol

Smoked fish products were processed at Lake Kenyatta, Mpeketoni area of Lamu County. Eight improved smoking ovens have been constructed to smoke fish products. Fish were purchased from fishermen in the area, gutted and cleaned thoroughly using potable water. The fish samples were then brined using 20% w/v of water for about one hour, removed and placed on the smoking trays to drain for 30 minutes. The smoking was done using a 4x8x3 feet double door smoking oven. About 3 kg of wood fuel was lit to burn completely till the flame was allowed to go off. Whole fish products were then transferred to the smoking ovens. The fish was then left to dry slowly in smoke and controlled heat (average 80^0C). Small quantities of wood fuel were added slowly to the fire until the product dried to below 20% moisture content. The process took 32 hours net drying period to completion. The final products were wrapped in aluminium foil, packed in trays and transported to KMFRI laboratory where shelf life studies were conducted.

Laboratory Analysis

Biochemical and sensory methods were used in the evaluation of quality changes with time. Samples for day 0 were analyzed immediately. About 30kg of the smoked products were used in shelf life study using different packaging methods separated in three lots. The first lot of 10 Kg samples were kept in open plastic containers (Open Air (OA)), the other 10 kg were packed in polythene papers and sealed (normal packaging (NP)) while the third lot were vacuum packed (VP). Subsequent biochemical and sensory analyses were carried out on day 15 and day 30.

mg malonaldehyde/kg = abs x 7.8

Where

abs = corrected absorbance of supernatant

7.8 = a constant

Biochemical and Sensory Determination

Determination of thiobarbituric acid reactive substances (TBARs)

The thiobarbituric acid-reactive substances (TBARS) assay was performed as described by Buege & Aust (1978). Ground sample (0.5 g) was homogenised with 2.5 ml of a solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25N HCl using homogenizer. The mixture was heated in a boiling water bath (95–100 ^{o}C) for 10 min to develop a pink colour, cooled with running tap water and centrifuged at 3600g at 25^{o}C for 20 min using a centrifuge. The absorbance of the supernatant was measured at 532nm. TBARS was calculated and expressed as mg malonaldehyde/kg sample as below.

Total Volatile Bases-Nitrogen (TVB-N)

The total volatile basic -nitrogen (TVB-N) was determined according to Kirk & Sawyer (1991). Approximately 5g of whole ground shrimp sample was homogenized with 15ml of 4% Trichloroacetic acid (TCA) (w/v) and centrifuged at 3000g for 3 minutes then filtered through filter paper (125mm diameter). A 5ml aliquot was removed and mixed with 5ml of 2M NaOH. The mixture was then poured into a semi-micro-distillation tube and steam distilled. The distillate was collected in a beaker containing 15 ml of 0.01M HCl standard to a final volume of 50 ml. Rosolic acid 1% in 10 ml (v/v) ethanol was used as indicator. The TVB-N was calculated using the following formula:

$$\text{TVB-N (mg/100g sample)} = \frac{Ml.\ of\ titrant \times 0.14 \times 2 \times 100}{Sample\ Wt.}$$

Determination of Proximate Composition

Protein analysis

Protein content in shrimp meat was determined based on Kjeldahl method (AOAC, 1990). A sample of 5 g was digested in Sulphuric acid (H_2SO_4) in the presence of copper sulphate as a catalyst. Thereafter, the sample was placed in the distillation unit, 2400 Kjeltec Auto Sample System. The acid solution was made alkaline by sodium hydroxide solution. Ammonia was then steam distilled in boric acid having indicators. The boric acid was then simultaneously titrated with 0.01M H_2SO_4. The nitrogen content was then multiplied by a factor of 6.25 to get the ratio of crude protein.

Determination of Fat Content

The fat content in shrimp flesh was determined using AOCS (1997) official method of analysis. The sample was extracted using petroleum ether, with a boiling range of 40-60°C. The extract was recovered using a Rotary evaporator. The extract was weighed and the fat content calculated as follows;

$$\%\ \text{fat content} = \frac{(weight\ of\ fat + container) - (weight\ of\ container)}{weight\ of\ sample} \times 100$$

Determination of Moisture Content

Moisture content was determined according to AOCS (1997) official method of analysis. In a pre-weighed aluminium foil, 5 g of crushed whole shrimp was dried for 24 hours in an oven at 105^{o}C and cooled in a desiccator to room temperature. The same was weighed and recorded accordingly. The moisture content was calculated as follows:

$$\text{Moisture content x (\%wb)} = \frac{initial\ Wt - \text{Final wt}}{initial\ Wt.} \times 100$$

Determination of Ash Content

The ash content was determined according to AOCS (1997) official method of analysis. 5g of shrimp flesh was dried for 24 hours in an oven at 105°C and cooled in a desiccator to room temperature. The same was weighed and recorded accordingly. In a known weight of aluminium foil, the samples were placed in the micro furnace at 450°C for six hours to ash completely. The ash content was calculated as follows:

$$\%\ \text{Ash content} = \frac{initia\ weight\ before\ ashing - \text{Final weight after ashing}}{initia\ weight\ before\ ashing} \times 100$$

Determination of Water Activity

Water activity was measured using the water activity meter (Decagon).

Temperature Measurements

Temperature humidity logger was used to measure the temperature.

Determination of Sensory Attributes

10 trained taste panellists were used to determine the sensory attributes using a 0-5 score range (5= best score and 0 = worst sore). The scores were added together and the average of all the four attributes (taste, appearance, texture and general appearance) used to determine its acceptability.

RESULTS AND DISCUSSION

Biochemical

There were appreciable changes in TVB-N for open and normal packaged products with no noticeable changes in the vacuum packaged ones. Open packaged samples had the highest result of 0.0046 ± 0.0003 mgN/100g on day 30. However, a different trend was seen in the TBARs results where only open packaging had an appreciable increase with the highest value being 5.7681±0.2300mg malonaldehyde/Kg on day 30 (Fig1&2).

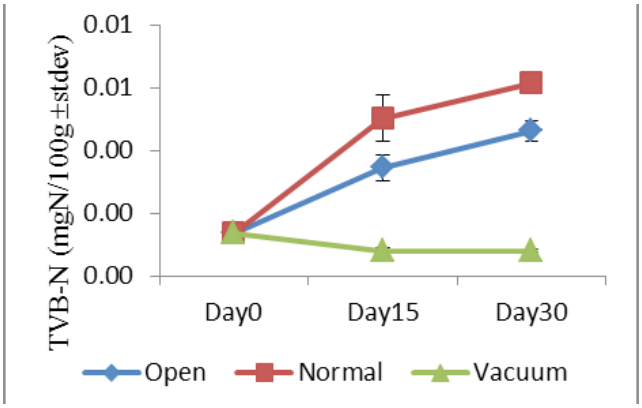


Fig 1: Changes in TBV-N during storage

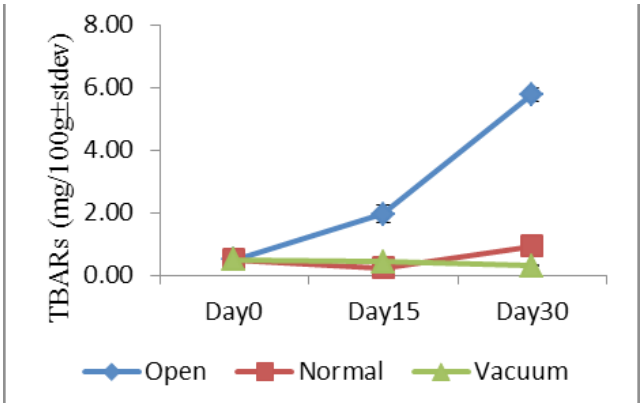


Fig 2: Changes in TBARs during storage

Proximate Composition

Percentage content of protein, fat, ash and moisture were determined for proximate composition during the 30 days period. Changes were noticeable in Open and Normal packaging with percentage of fat appreciably decreasing from 5.8169 ±0.1956 on day 0 to 4.9742±0.1582 and 5.8169±0.1956 to 3.9754±0.1735 on day 30. There was not much change noticed in percentage of ash content during the 30 days storage period. However, moisture content for open packaging increased from 18.7084±0.6191 to 24.9475±0.9362 during the storage period while protein behaved in the reverse by decreasing from 72.996±1.2976 to 60.8362±2.1406 for the open package. No major changes were noticed on protein and moisture content for the 30 days storage period (Figs 5 & 6).

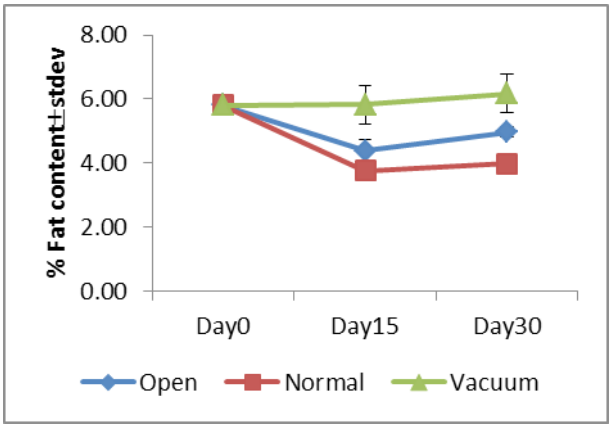


Fig 3: Changes in percentage fat content during storage

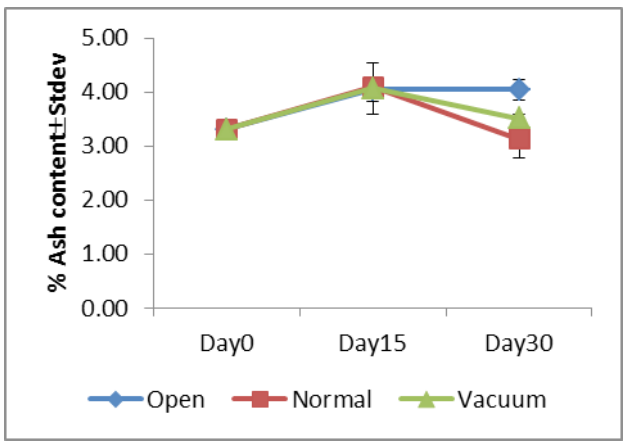


Fig 4: Changes in percentage ash content during storage

Fig 5: Changes in moisture during storage

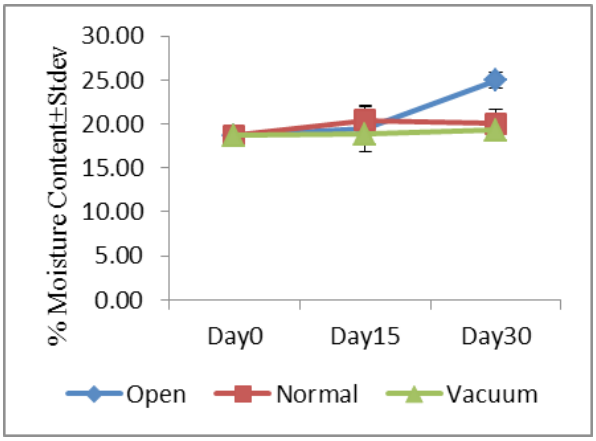
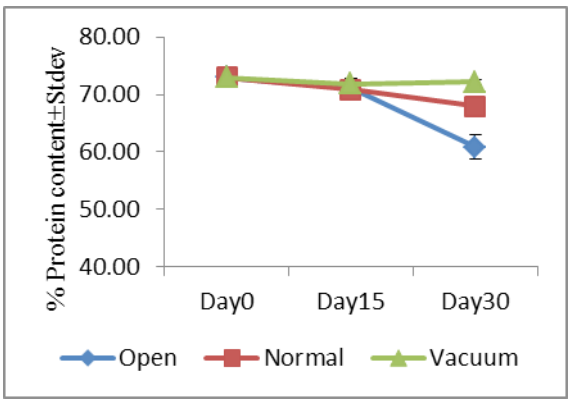


Fig 6: Changes in protein content during storage



Linear Regression Results

A multi linear regression analysis was performed on the biochemical and proximate composition parameters to establish the relationship between storage period with changes in parameter levels. TVB-N showed significant linear relationship in all the packaging conditions hence affected directly with storage period under all conditions. However, under vacuum packaging the effect is appreciably reduced (r=0.83) compared to open (r=0.97) and normal packaging (r=0.94). This could be attributed to reduced chances of samples being in contact with open air exposed to microorganism activities. TBARs showed strong linear changes with time in both open (r=0.96) and vacuum packaging (r=0.86). However, under normal packaging there was no significant linear relationship with storage period. Ash and moisture content results showed no linear relationship with storage period except for the open packaged samples. This could be associated with reduced chances of being in contact with atmospheric moisture due to sealed packaging. Protein content results had significant linear reduction with storage on both open (r=0.90; $p<0.05$) and Normal packaging (r=0.86; $p<0.005$). This could be associated with continued microorganism activity on the free amino acids to protein products due to air contact with the samples even after packaging. Fat content did not show significant linear relationship in open and vacuum packaging except on the normal packaging where there was a relationship (r=0.86; $p<0.05$). This could be attributed to continued lipid oxidation due to air in the package emitting non fatty acid products.

Table 1: Multi-linear regression of biochemical and proximate composition of the products during the storage period under different packaging conditions

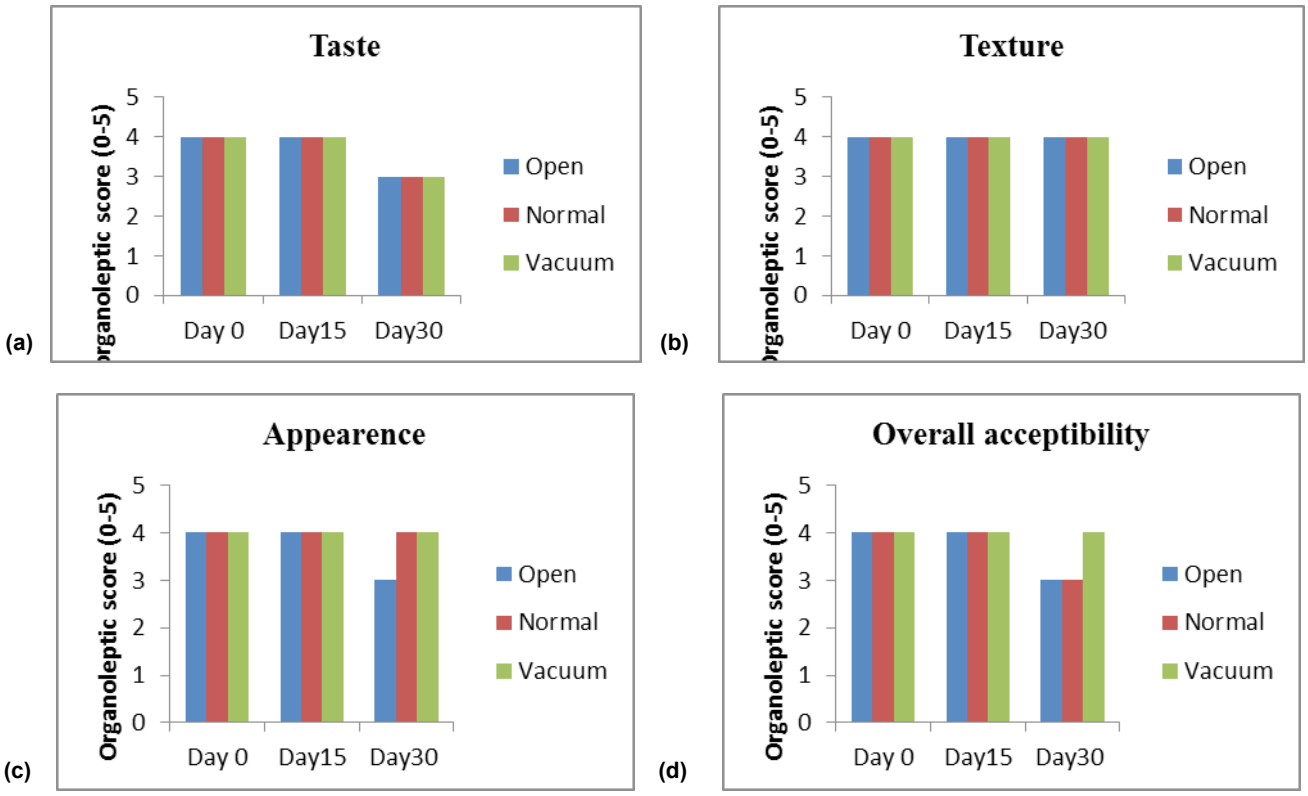
Parameter	Storage condition	Day 0	Day15	Day 30	R	P
TVB-NmgN/100g	Open	0.0013 . 0.0001	0.0034 . 0.0004	0.0046 . 0.0003	0.9704	0.0000
	Normal	0.0013 . 0.0001	0.0050 . 0.0007	0.0061 . 0.0003	0.9404	0.0002
	Vacuum	0.0013 . 0.0001	0.0008 . 0.0001	0.0008 . 0.0000	0.8354	0.0051
TBARs mg/Kg	Open	0.4940 .0.0596	1.9559 .0.2833	5.7681 .0.2300	0.9655	0.0000
	Normal	0.4940 .0.0596	0.2366 .0.0162	0.9277 .0.0753	0.6129	0.0792
	Vacuum	0.4940 .0.0596	0.4143 .0.0821	0.2897 .0.0201	0.8583	0.0031
% Protein(N)	Open	72.996.1.2976	71.0556.1.6716	60.8362.2.1406	0.8993	0.0010
	Normal	72.996.0.9739	70.8531.1.0251	67.9204.1.6917	0.8618	0.0028
	Vacuum	72.996.1.2976	71.8136.0.9263	72.1527.0.3865	0.3745	0.3207
% Fat	Open	5.8169 .0.1956	4.3810 .0.3626	4.9742 .0.1582	0.6315	0.0681
	Normal	5.8169 .0.1956	3.7566 .0.2176	3.9754 .0.1735	0.8016	0.0094
	Vacuum	5.8169 .0.1956	5.8178.0.6068	6.1766.0.6096	0.3271	0.3902
% Ash	Open	3.3121 .0.1611	4.0430 .0.2230	4.0468 .0.1879	0.7904	0.0112
	Normal	3.3121 .0.1611	4.0911 .0.1091	3.1262 .0.3446	0.1658	0.6700
	Vacuum	3.3121 .0.1611	4.0651 .0.4821	3.5106 .0.0719	0.20225	0.6013
% MC	Open	18.7084 .0.6191	19.4351.2.6517	24.9475.0.9362	0.8220	0.0066
	Normal	18.7084 .0.6191	20.4197.1.4887	20.0137 .1.5901	0.1847	0.6342
	Vacuum	18.7084.0.6191	18.8803.0.9267	19.3138.0.4053	0.4023	0.2830

The values in bold show significant linear relationship with storage time

Sensory Evaluation

Sensory evaluation method was used to determine the level of acceptability by the consumers during the storage period. Taste, texture, appearance and overall acceptability were scored at a scale of 0-5 and the averages used as indicators of acceptability of the products.

Fig. 7: Changes of sensory attributes with time during storage period



All the sensory attributes did not show any significant different in panellists' response with all panellists scoring 4 out of 5 indicating higher acceptability of the product. However, day 30 had a slight drop (score 3) in taste and appearance.

Water Activity and Temperature

The water activity (Wa) and temperature were stable throughout the storage period. However, the vacuum packaged samples showed higher values with storage period. Temperatures were stable throughout with insignificant changes during the period (Fig. 8 & 9).

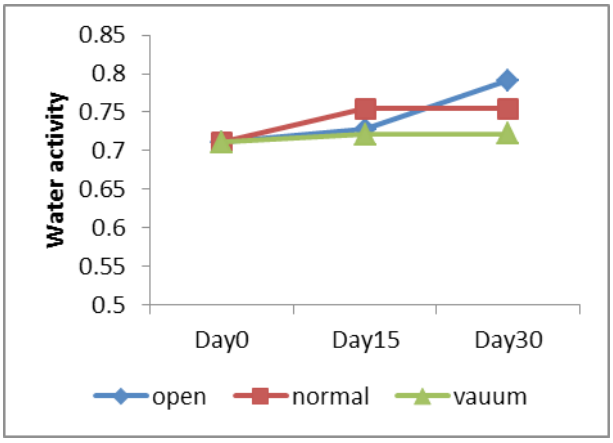


Fig 8: Water activity during storage period

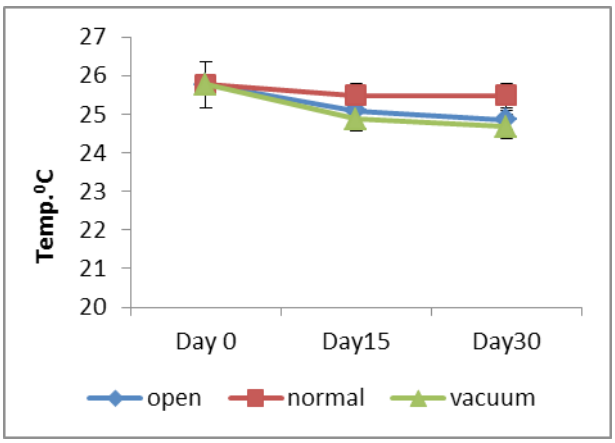


Fig 9: Temperature during storage period

CONCLUSION AND RECOMMENDATION

Biochemical Evaluation

All biochemical parameters showed significant changes for open storage conditions. This means that open packaging reduces the shelf life significantly as all parameter showed significant linear relationship with time. Normal and vacuum packaged sampled had the least effect during storage period although the vacuum was the best in maintaining quality. It was observed that using vacuum packaging would extend the shelf life of smoked products significantly. However none of the stored samples exhibited critical levels. Sensory results showed that all the samples within acceptable levels. Amongst the proximate composition parameters, samples stored under open packaging are affected most as all showed linear changes with time. Therefore, samples are affected most under open packaging.

Vacuum packaging is recommended for packaging of smoked fish samples. However, storage trials should be done for longer period than 30 days in order to establish the critical storage limits at which the parameters reach the unacceptable levels. Development of smoked fish product needs to be tested in the market using vacuum packaging.

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Our acknowledgement goes to the Kenya Caatal Development Project (KCDP) for having funded this study for the entire period. We also appreciate KMFRI for having supported this work by providing workspace. Our gratitude also goes to the technical staff of the Kenya Marine and Fisheries Research Institute (KMFRI), various Beach Management Units (BMUs) and all those who assisted in one way or the other to make this work a reality.

REFERENCES

AOAC. (1990). Official methods of analysis of the association of official analytical chemists Arlington. Association of Official Analytical Chemists Inc, (5(1):), p 771.

AOCS. (1997). Sampling and analysis of oil seed by-products. (with modification according to application note tecator no AN 301). Official Method, pp3-38.

Buege, J., & Aust, S. (1978). Methods Enzymol, (52), 302–310.

Chaijan, M. (2011). Physicochemical changes of tilapia (Oreochromis niloticus) muscle during salting. Food Chemistry, 129(3), 1201–1210.

Kirk, S., & Sawyer, R. (1991). Pearson's composition and analysis of foods. Longman Group Ltd.

Oyero, J. O., Sadiku, S. O. E., & Eyo, A. A. (2012). The effect of various smoking methods on the quality of differently salted Oreochromis niloticus. Internasional Journal of Advanced Biological Research, 2(4), 717–723.

Piggott, G. M. (2002). Smoking of fish, special considerations. Institute for Food Science and Technology University of Washington.

Evaluation of UV-Resistance of Epibiotic Bacteria Co-existing with the Kenyan Marine *Lyngbya majuscula*

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Abstract

The marine cyanobacterium *Lyngbya majuscula* is reported to be a source of a wide array of natural products. Some of the products exhibit photo-protective properties. L. *majuscula* has also been shown to live in association with epibiotic bacteria. However, the potency of these epibionts to withstand ultraviolet radiation is not well documented. This study focused on epibiotic bacteria isolated from the surface of *L. majuscula* from Kenya. Twelve strains or isolates were exposed to UV irradiance (365 nm, intensity of 11.6W cm^{-2}) for 15, 30 and 45 minutes. Survival curves showed that the *Bacilli strains* were the most tolerant to UV, followed by the β-*proteobacteria*, while the actinobacteria were the least resistant to UV. The observation that the actinobacteria were less resistant to UV suggested that cell wall characteristics and G+C content are not the sole determinants of UV resistance. It would be interesting to determine the compounds and/or metabolite underlying the ability of these isolates to withstand exposures of varying levels of UV-radiations.

Key words: Lyngbya majuscula, epibiotic bacteria, Ultraviolet radiation

INTRODUCTION

Cyanobacteria are Gram-negative, photolysis-mediated oxygen evolving, cosmopolitan prokaryotes that have survived and flourished on earth for over two billion years with the creation of oxygenic environment (Sergeev et al., 2002). They have become recognized as an extremely rich source of novel, bioactive secondary metabolites with approximately 700 different natural compounds having been isolated and characterized (Tidgewell et al., 2010a). Some of these compounds have gained considerable attention due to their pharmaceutical and biotechnological potential (Tan, 2007). Marine strains of the genus *Lyngbya* are the most prolific producers of these natural products with nearly 300 compounds reported from this genus (Tidgewell et al., 2010a), with 76% of these products attributed to the species *L. majuscula*. The compounds isolated from *L. majuscula* include those which exhibit biological activities which may have use in human health including anticancer, anti-inflammatory, antibacterial and anti-infective therapeutic agents (Tidgewell et al., 2010b). Additionally, the species have been reported to be potential sources for photo-protective agents such as scytonemin and mycosporine-like amino acids (MAAS) which are known to screen-out harmful effects caused by ultraviolet (UV) radiation (Cockell & Knowland, 1999). Marine *Lyngbya* isolates are found pan-tropically in shallow Coral reef environments with frequent exposure to diverse natural selection pressures such as desiccation during low tide and exposure to high fluxes of UV radiation (Gerwick et al., 2008). They are known to grow in close association with other cyanobacteria and algae, and provide an ideal substrate for a variety of heterotrophic bacteria (Simmons et al., 2008). Epibiotic bacteria in general are heterotrophic (Hempel et al., 2008; Hube et al., 2009). These heterotrophic bacteria can be found attached to cyanobacterial trichomes as well as imbedded in the mucopolysaccharide layer surrounding the trichomes (Nausch, 1996) of unicellular cyanobacteria (Brunberg, 1999). Many of the heterotrophic bacteria play an important role in nutrition (Hempel et al., 2008), and defence against predators and biofouling (Bewley et al., 1996). However, the existence of epibiotic bacteria among *L. majuscula* collected from the Kenyan Coast responses towards varying levels of Ultra-violent radiations are not well understood. The aim of this study was to evaluate UV resistance of the epibiotic bacteria co-existing with the Kenyan marine *L. majuscula.*

MATERIALS AND METHODS

Experimental Layout

The bacterial strains used in this study were previously isolated from the Kenyan marine *L. majuscula* collected from four different sites along the Kenyan Coast namely Mida (039.99505º to 039.96600ºE) and Kilifi (039.785˚E to 039.835˚E) in the North Coast and Shimoni (039.36565ºE to 039.36696ºE) and Wasini (039.35906ºE to 039.35942ºE) in the South (Table 1).

Irradiation was done by 'direct plate – kill' method following a slight modification of Kevin and Alice (2001). Cultures for irradiation were grown on nutrient agar media plates and incubated overnight at 30ºC. A suspension of the overnight culture was made using sterile distilled water and serial dilutions were prepared up to 10^{-3}. The 10^{-3} dilutions were used

for preparing the plates for irradiation by spread-plating 100µl of this dilution onto nutrient agar petri plates. After plating, the plates were irradiated in a custom-built UV analysis cabinet (ACM 82307-Delhi (India) using the long wavelength lamp (365nm-UVA, intensity 11.6W cm^{-2}). Different exposure times of 0, 15, 30 and 45 minutes were used, with the zero (0) minute exposure time being used as control. All irradiations were done in the dark to avoid photo-activation and light source was only present during the transfer of the petri plates containing the bacterial treated media into and out of the UV chamber. The lids of the treatment plates were also removed before placing the plates into the chamber to avoid shielding by the lid and replaced immediately after irradiation. The treated plates were then incubated in the dark at 30ºC for 24 to 48 hours before scoring the number of colonies. The UV dose (in W S^{-1} cm^{-2}) was calculated by multiplying the intensity by the irradiation time (in seconds). The UV intensity was considered to represent the irradiance on a flat surface.

Table 1: Bacterial isolates used in the experiment

	Isolate	Closest relative	% similarity	Group	Site isolated
1	79T	*Alcaligenes faecalis* strain IAM 12369	87	β-proteobacteria	Wasini
2	75W	*Bacillus anthracis* str. Ames strain	85	Bacilli	Shimoni
3	74O	*Alcaligenes faecalis* subsp. phenolicus strain J	90	β-proteobacteria	Shimoni
4	85CW	*Bacillus aerius* strain 24K	93	Bacilli	Wasini
5	62M	*Microbacterium koreense* strain JS53-2	97	Actinobacteridae	Mida
6	70CW	*Paenibacillus taichungensis* strain BCRC 17757	99	Bacilli	Shimoni
7	70W	*Alcaligenes faecalis* strain NBRC 13111 16S	91	β-proteobacteria	Shimoni
8	72T	Bacillus anthracis str. Ames strain Ames	87	Bacilli	Shimoni
9	84CY	*Bacillus marisflavi* strain TF-11	99	Bacilli	Wasini
10	72CW	*Bacillus aerius* strain 24K	96	Bacilli	Shimoni
11	KO	*Exiguobacterium* sp. AT1b strain AT1b	99	Bacilli	Kilifi
12	82W	*Bacillus anthracis* str. Ames strain Ames	88	Bacilli	Wasini

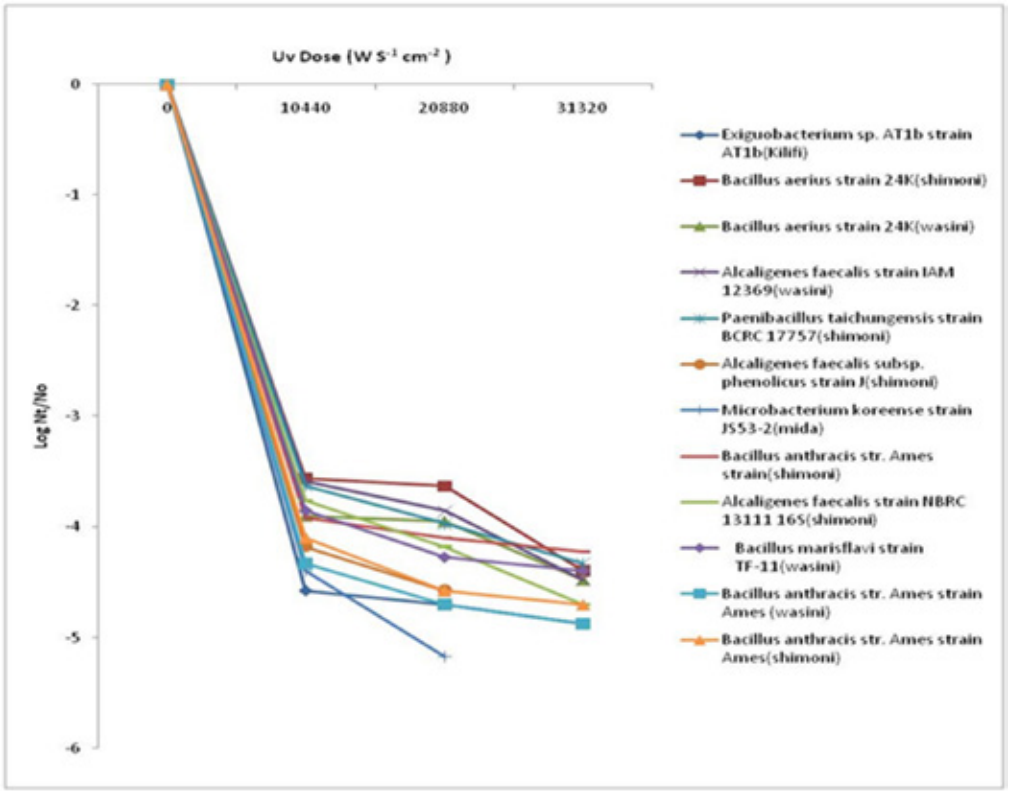

Fig. 1: UV sensitivity curves for the bacterial isolates. Nt number of CFU at time t, No is number of CFU at time 0.

RESULTS AND DISCUSSION

The responses of the isolates towards UV exposures varied greatly as shown in **Fig. 1**. Of the tested isolates, the bacilli group was the most resistant to UV. All the isolates were gram positive. This finding corroborates with the suggestion that Gram-positive bacteria are better adapted to UV stress because their cell walls screen out a considerable fraction of UV radiation (Jagger, 1985). The resistance of these isolates varied with site of isolation as those from Shimoni were more resistant, followed by Wasini, and the least were from Kilifi. The variation in resistance amongst the sites could have been contributed to other factors such as human activities closer to the collection site .

The actinobacterium tested in this study (*Microbacterium koreense* strain JS53-2 was the least resistant to the UV, which is in agreement with observations by Ordon˜ez et al. (2009), demonstrating that cell wall characteristics and G+C content are not the sole determinants of UV resistance. However, this observation was in contrast to that made by Warnecke et al. (2005) where the high G+C content of Actinobacteria is proposed to confer protective adaptation against UV radiation by minimizing the formation of cyclobutane dimmers.

In the present study, beta-proteobacteria tested exhibited medium resistance to UV compared to the other groups. These finding corroborates with Ordon˜ez et al. (2009), where the beta-proteobacteria studied had medium resistance to UV-B exposure.

CONCLUSION

The results of this study have revealed that different epibiotic bacteria found on the surface of the Kenyan marine *L. majuscula* exhibit varied responses to UV exposure. There is need to analyze and understand the different compounds/metabolites associated with resistance to UV and also the mechanisms of UV tolerance.

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REFERENCES

Bewley, C. A., Holland, N. D., & Faulkner, D. J. (1996). Two classes of metabolites fromTheonella swinhoei are localized in distinct populations of bacterial symbionts. *Experientia*, *52*(7), 716–722.

Brunberg, A.-K. (1999). Contribution of bacteria in the mucilage of Microcystis spp.(Cyanobacteria) to benthic and pelagic bacterial production in a hypereutrophic lake. *FEMS Microbiology Ecology*, *29*(1), 13–22.

Cockell, C. S., & Knowland, J. (1999). Ultraviolet radiation screening compounds. *Biological Reviews*, *74*(3), 311–345.

Gerwick, W. H., Coates, R. C., Engene, N., Gerwick, L., Grindberg, R. V., Jones, A. C., & Sorrels, C. M. (2008). Giant marine cyanobacteria produce exciting potential pharmaceuticals. *Microbe-American Society for Microbiology*, *3*(6), 277.

Hempel, M., Blume, M., Blindow, I., & Gross, E. M. (2008). Epiphytic bacterial community composition on two common submerged macrophytes in brackish water and freshwater. *BMC Microbiology*, *8*(1), 58.

Hube, A. E., Heyduck-Söller, B., & Fischer, U. (2009). Phylogenetic classification of heterotrophic bacteria associated with filamentous marine cyanobacteria in culture. *Systematic and Applied Microbiology*, *32*(4), 256–265.

Jagger, J. (1985). Solar-UV actions on living cells.

Lin, K., & Wang, A. (2001). UV mutagenesis in Escherichia coli K-12: Cell survival and mutation frequency of the chromosomal genes lacZ, rpoB, ompF, and ampA. *J Exp Microbiol Immunol*, *1*, 32–46.

Nausch, M. (1996). Microbial activities on Trichodesmium colonies. *Marine Ecology Progress Series*, *141*, 173–181.

Ordoñez, O. F., Flores, M. R., Dib, J. R., Paz, A., & Farías, M. E. (2009). Extremophile culture collection from Andean lakes: extreme pristine environments that host a wide diversity of microorganisms with tolerance to UV radiation. *Microbial Ecology*, *58*(3), 461–473.

Sergeev, V. N., Gerasimenko, L. M., & Zavarzin, G. A. (2002). The proterozoic history and present state of cyanobacteria. *Microbiology*, *71*(6), 623–637.

Simmons, T. L., Coates, R. C., Clark, B. R., Engene, N., Gonzalez, D., Esquenazi, E., … Gerwick, W. H. (2008). Biosynthetic origin of natural products isolated from marine microorganism–invertebrate assemblages. *Proceedings of the National Academy of Sciences*, *105*(12), 4587–4594.

Tan, L. T. (2007). Bioactive natural products from marine cyanobacteria for drug discovery. *Phytochemistry*, *68*(7), 954–979.

Tidgewell, K., Clark, B. R., & Gerwick, W. H. (2010). The natural products chemistry of cyanobacteria.

Tidgewell, K., Engene, N., Byrum, T., Media, J., Doi, T., Valeriote, F. A., & Gerwick, W. H. (2010). Evolved

diversification of a modular natural product pathway: apratoxins F and G, two cytotoxic cyclic depsipeptides from a Palmyra collection of Lyngbya bouillonii. *ChemBioChem*, *11*(10), 1458–1466.

Warnecke, F., Sommaruga, R., Sekar, R., Hofer, J. S., & Pernthaler, J. (2005). Abundances, identity, and growth state of actinobacteria in mountain lakes of different UV transparency. Applied and Environmental Microbiology, 71(9), 5551–5559.

Identification and Characterization of Kenyan Marine Microalgae Strains towards Bio-fuel Production

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Abstract

The rising global energy demand in both developed and developing countries is heavily straining the limited energy reservoir. The increased use of fossil fuels result in large Green House Gases (GHS) emissions, that are usually considered the main cause of global warming. Microalgae are projected to be the source for the third generation biofuel. Microalgae are one of the most abundant organisms present on the planet and seem to have received more attention due to their lipid reserving capacity. The purpose of this study was to seek, identify and characterize microalgae from the Indian Ocean and its environs which included; polluted sites; tenable for biofuel production. Six stations (Mikindani, Moroto, Fort Jesus, Kenya Meat Commission, Coast General Hospital, Technical University of Mombasa) were selected based on their; physic-chemical parameters. Sixty four (64) strains of microalgae were obtained in five stations through morphological techniques. On further isolation, 19 strains were independently secluded and their *in vitro* cultures established. The microalgae were cultured in the laboratory conditions in three media namely Walne, F/2 and TAP. Algal growth parameters i.e., pH, optical density (OD), dry and chlorophyll-a (Ch-a), were measured at zero-time and at the end of the experimental period. The results revealed that media preference for overall microalgae growth was 38% in F/2 media, 31% in Walne and 30% in TAP media. The *Oscillatoria* genera from all the stations were found to exhibit 100% growth in all the media. From the results, the suitable candidate strains recommended for mass cultivation for mass biodiesel production exploitation include *Oscillatoria* genera because of their fast growth and ability to withstand varying physicochemical conditions of *in vitro* culture.

Keywords—Biodiesel, Biofuel, Microalgae, Oscillatoria.

INTRODUCTION

Biofuel production from renewable sources is widely considered a sustainable energy alternative source compared to petroleum. Microalgae provide a viable means of environmental and economic sustainability (Dragone et al. 2010). Microalgae currently hold promise as an ideal third generation biofuel feedstock. This is supported by their rapid growth rate, carbon dioxide fixation ability and high lipid production capacity. They also do not compete with food crops for arable land and water resources for irrigation. They can potentially be produced on non-arable land (Dragone et al., 2010). Some microalgae strains are capable of generating 70% weight by weight (w/w) lipids in their biomass (Alcain, Anna, & Kushiga, 2010).However; they may yield significant lipid content under stress conditions. Some of the stress causing factors include; nutrients, light, salinity among others. Utilization of microalgae for biofuel production is meritorious over higher plants since they synthesize and accumulate large quantities of neutral lipids; they are also capable of all year round production yielding higher oil quantities (Abubakar & Mutie, 2012).Further, Chisti (2007) advances that microalgae need less water than higher crops, hence reducing the load on freshwater sources.

Microalgae are reported to exhibit faster growth rates besides expressing the ability to grow under saline conditions that are unsuitable for agriculture (Alcain, Anna, & Kushiga, 2010).Adoption of biodiesel will support environmental conservation, agricultural and economic developmental goals. However, a successful and economically viable algal based oil industry depends on the selection of appropriate algal strain. Species of microalgae should therefore be bio prospected to determine suitability for oil production (Araujo, Matos, Gonçalves, Fernandes, & Farias, 2011). In the last decade, Deoxyribonucleic Acid (DNA) sequencing and genomics have brought substantial changes to microalgae taxonomy. Both the subunit ribosomal DNA and its genes have been used in studies for species identification because they include the highly conserved regions at the species level.

MATERIALS AND METHODS

Description of Study Site

Sampling was done three times at the Tudor Creek. Tudor Creek is located along Latitude of 4.0000°, Longitude 39.6500°. It is 15.5km away from Mombasa town to the North. The study site was accessed by a fibre glass boat at the Tudor Water sports entrance. Sampling was done in triplicates; one set was preserved with 5% lugol's iodine for quantitative diversity studies. The second set was isolated and cultured, while the third set was incubated at 28^{0}C for fourteen days. Temperature, pH, total suspended solids and salinity measurements for all the sampled stations were recorded.

Sampling of microalgal strains

Water samples were collected in triplicate at the sampling stations for analysis. Surface water samples were collected using a bucket. 40 litres of water was passed through 20µm mesh-size

plankton net for concentration to 50ml. The resultant concentrated plankton was transferred to sample bottles labeled with date and sampling station, and preserved in 5% Lugol's solution. Qualitative samples were stored in a cooler box after collection and transported to the laboratory for further analysis.

Water Quality Parameter

Surface water quality parameters were measured *in situ* at each station; pH was measured using an electronic pH probe, temperature was measured using the YSI Model 550A, salinity was measured using a hand-held refractometer.

Quantitative Sample Analysis

In the Laboratory, 1ml aliquots of samples preserved in Lugol's iodine solution were mounted on slides and observed under an inverted microscope and the counts of all seen phytoplankton recorded. The algal species were identified using the identification manual of marine microalgae by Hasle & Syvertsen (1977).

Isolation and Purification

The algal samples for qualitative analysis were subjected to purification by serial dilution, addition of antibiotics, and addition of enrichment media followed by culturing them in TAP, F/2 and Walne media for microalgae and incubated at 25 ± 1°C under 1.2+0.2 k lux intensity with 16:8 hours light photoperiod. The purity of the culture was ensured by repeated sub culturing in fresh media and regular observation under the inverted microscope.

Identification of Microalgal Strains

The purified monoalgal samples were observed under the inverted microscope and the morphological properties of the isolates identified based on the manuals.

Culture Maintenance

Unialgal cultures of the microalgae strains were maintained in the F/2 and Walne culture media. A drop of the respective culture was inoculated aseptically using a sterile micropipette into 50 ml of the medium in sterile 50 ml conical flasks. The cultures were incubated in an algal growth room with constant illumination at 110 µmol.m-2/s at 25°C. This procedure was performed on weekly a basis.

Isolation and Purification of Microalgal Cultures

This was done through isolation of the microalgae and culturing them on solid and the three liquid media namely; TAP, Walne and F/2

Measurement of Growth Rate

The growth rate of algae was measured by optical density at 660nm, 680nm, 760nm and 780nm for 4weeks. Daily measurements were also taken for 7 days.

Identification of Lipid Producing Microalgae Strains

Nile red staining was conducted to detect intracellular lipid droplets. Microalgae cells (0.5 ml) were collected by centrifugation at 1,500 rpm for 10 minutes and washed with physiological saline solution (0.5 ml) several times. Thereafter, the collected cells were resuspended in the same solution (0.5 ml). Nile red solution (0.1 mg/ml in acetone) was added to cell suspensions (1:100 v/v) and incubated for 10mins. After washing once, stained microalgae cells were observed by Fluorescent microscopy. Microscopic photographs were taken with a Nikon E600microscope

Determination of Oil Content Microalgae

One ml of growing algae in F/2 media was collected in 3 replicates in 1.5ml tubes and centrifuged at 13000rpm for 5 minutes. The pellets were frozen in liquid nitrogen and stored at -80^{0}C. Thereafter the pellets were measured to determine the weight. The lipids were extracted as follows; in the frozen pellets, 200µl of a mixture of chloroform: isopropanol (1:1) was added and vortexed vigorously for 3 minutes. They were then centrifuged using an electronic centrifuge(model 80-1) at 13,000rpm for 5minutes .The supernatant was transferred to a new tube, and then the pellets were re-extracted with 500µl of hexane and; vortexed vigorously for 3 minutes. The samples will then be centrifuged and the supernatants combined. The supernatants were dried by an evaporator and the amount of lipids measured gravimetrically .The Bligh and Dyer method of extraction using chloroform and methanol was also used (Bligh & Dyer, 1959).

RESULTS AND DISCUSSIONS

Physico-chemical Characteristics

The mean surface water temperature was 27.75^{0}C with a standard deviation of 0.5 and standard error of 0.25. The highest average temperature for the Creek was 31^0 C while the lowest average temperature was 21^{0}C (Table 1). Analysis of variance reveals that there was no statistically significant variation in Water and Temperature between sampling stations and between the three months of sampling at (P>0.05) For the pH was observed during the study period, with the highest average value of 8 and a lowest of 7.8. The mean ± SD for pH is 7.25 ± 0.23 (Table 1). This observed variation was statistically significant. The surface Water Temperature ranged from 27^{0}C to 28^0 C. The highest temperature was recorded at Coast General Hospital station and Technical University while the lowest temperature was recorded at Mkomani station. The pH was observed during the study period, with the highest average value of 8.0 and a lowest of 7.8. The salinity ranged from 34 - 36.3 PSU. With the highest concentration at Mkomani station. Total Suspended solids ranged from 0.037-0.162g/l with the highest concentration being recorded at Fort Jesus.

Table 1: Physico-chemical parameters of the sampled stations

Parameter (Units)	TSS (g/l)	Salinity (psu)	Temp (°C)	Ph
Mean	0.07	35	27.75	7.85
Standard Error	0.03	0.48	0.25	0.05
Median	0.05	34.85	28	7.8
Standard Deviation	0.06	0.96	0.5	0.1
Variance	0.003	0.92	0.25	0.01
Range	0.125	2.3	1	0.2
Maximum	0.162	36.3	28	8
Minimum	0.037	34	27	7.8

Quantitative Sample Analysis

This was determined by observing the samples preserved in lugol's iodine under the Leica Inverted microscope.

Species Present

The species were identified morphologically using conventional means by use of the phytoplankton guides. The species observed are shown in table 2. The species present in all the seven stations were; *Oscillatoria* spp., *Thallasiosira* sp., *Coscinodiscus* sp., and *Nitzschia*. The species present in only one station were; Monidiscus sp, *Coscinodiscus* sp, *Protoperidinium* sp, Dictyliosolenia sp, Bleaklayer sp., Protocentrum sp., Hemidiscus sp, Anabaena sp., *Dactyliosolen fragilissimus*, *Cylindrotheca closterium*, *Gonyaulax spinifera*, Spatulodinium sp., *Nitsia longissima*, *Anacustis nidulans*.

The most abundant phytoplanktons are in the group of Bacilliarophyta (diatoms) which occupied 60% followed by the Cynanophyta (cyanobacteria) which occupied 30% and chlorophyta occupying 10%. Bacillariophyta have been reported by many authors to be dorminat in the phytoplankton composition as it is in the present study (Polat & Aka, 2007). Chlorophyta was the second group after Bacillariophyta in the number of identified species, these result also agree with study on Grand River in Oklahoma by (Pfiester et al.,1980). It is also in agreement with other studies in Iraq (Al-Handal, Al-Assa, & Al-Mukthar, 1989). The maximum occurrence of phytoplankton was in October 2014 and thereafter it decreased. This may be due to available nutrients and other physical and chemical factors which promote growth of phytoplankton. While the minimum total number of phytoplankton species was recorded at Moroto which might be due to domestic discharge and effluents from run-off that empty into the river. This corresponds to the work of (Hassan et al.,2008).The differences in number of taxa and number of individuals between sampling stations for each class of phytoplankton may be due to differences in temperatures and pH. According to Wilhm and Dorris, (1966),species obtained at different pH and temperatures have suggest a relationship between species diversity and pollution status of aquatic system and classified as follows; > 3 = Clean water, 1-3 = moderately-polluted < 1 = Heavily polluted. Water pollution levels could also be accurately identified by analyzing the species abundance, physiological, biological responses and residue contents (Chen et al 2011). However, algae may not be only significant for biomonitoring studies but could also be a useful phytoremediation technology to restore water quality due to their high bioaccumulation ability

Shannon Wiener Diversity Index

Computation of diversity index was also done. Resultant graphs were drawn as Shown in Fig.1. From the results shown;

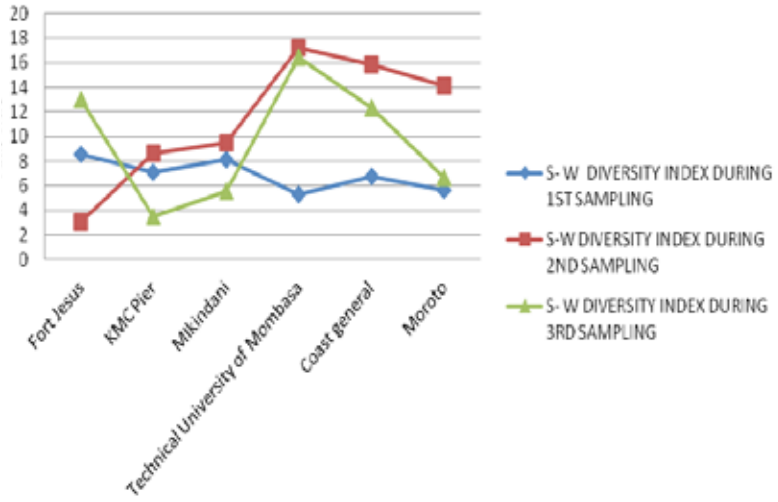

Fig. 1: Comparative diversity indices of the sampled stations

Phytoplankton Abundance and Diversity

Diversity indices reveal that TUM station had the highest index of 16.47. For all the diversity was stable. This indicated a large abundance of phytoplankton in the marine ecosystem sampled.

The highest diversity occurred during the second sampling which indicates very high diversity indices from the graphs shown.

Liquid Media Preferences

Species occurrence was highly dependent on the media, the media used were TAP, Walne and F/2. Plots of media and the species obtained are shown in Fig.2

Solid Media Preferences

The three media namely TAP; Walne and F/2 were prepared and solidified with Agar. Samples from all the four stations were inoculated. Incubation was done at 27°C for two weeks with 12:12 light /dark .

Streak Plate Method

Two weeks after initial incubation, most of the plates showed colonial formations. Some plates also showed strains growing beneath the surface. For subsequent sub culturing rounds, microorganisms showed growth after only seven days in the agar

surface, probably due to acclimatization in the new conditions. Even though single colony picking and subculturing was done with extreme care, many of the new plates still showed mixtures of two organisms. After approximately three subculturing rounds, most plates showed organism uniformit In few cases, however, it was not possible to get rid of a round, colonial, transparent microorganism that appeared in the agar two or three weeks after incubation

Serial Dillution

The following microalgae were isolated and cultured through serial dilution.They were observed under the digital microscope (Motic DM 111) and their photographs taken.

Culture of Microalgae in Liquid Media

The isolated microalgae were then cultured in test tubes and conical flasks of 250 ml with the suitable media being supplied to each of them. Continuous and vigorous aeration was provided to culture which keeps the culture in suspension. In addition continuous aeration is also helpful for uniform distribution of nutrients.

From the graph it shows tha F/2 and Walne Media are the most prefered media for growth of most microalga

Total Isolates Obtained

At the end of the isolation process, there were various species obtained. Isolation was done based on stations, and species. The methods used for isolation included; serial dilution, culturing in liquid and solid media. The isolation protocol developed in-house became an efficient method for the microalgae isolation and transfer from the natural environment in to laboratory conditions. The streak plate method for microalgae enrichment, although slow, proved to be an excellent approach for the isolation of green phototrophic microorganisms. With regards to the physical properties of the water samples, they remained fairly constant all throughout the different depths assessed as well as in the different locations.

Temperatures fluctuated between 20.5 and 23.5^{0}C, pH between 6.9 and 8.1, and salinity between 32 and 37.8 psu.

Regardless of the stations the microalgae colour appearance on the solid media included; 15% green, 16% brown, 8% pink, 23% cream. These results corresponds with the study from (Wilhm & Dorris, 1966).Isolation procedures using solid media allowed for the selection of microorganisms with colors besides green; culture attempts of the same in liquid media were utterly unsuccessful. None of the solid or liquid media were prepared with an external carbon source, with the purpose of selecting phototrophic organisms. However, solid media was gellied with agar, which is a polymer of the sugar galactose.

However, certain marine organisms have the capacity of producing agarose and liquefy solid media efficiently (Chen, Sommerfeld, & Hu, 2011).These enzymes are responsible for allowing them to use agar as their primary carbon source and enables their ability to thrive in the ocean .In this case, it might have happened that the organisms isolated, other than the green ones, had this innate ability to metabolize agar and thus developed efficiently in solid media; interestingly, all of them showed growth close to the agar surface.

As for the liquid Media preference, F/2 Media with silicate seemed to be the most preferred because there was a large growth of the species. TAP media was the least preferred probably due to the fact that it forms a cloudy appearance after a short while. These results are consistent with observations made by (Pfiester, Lynch, & Wright, 1980) and many others who noticed the zonation of pigments on marine organisms depending on depth.

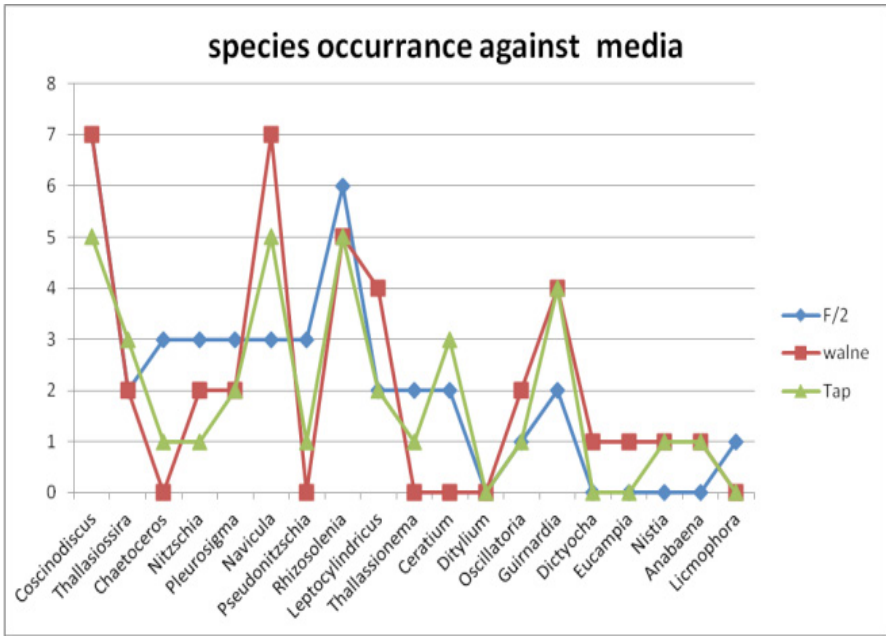

Fig. 2: Line graph showing a graph of media against media

Measurement of Growth

Optical density was taken at intervals of one week. The absorbance readings were taken at three optical densities of; 620nm, 650nm and 750 nm. The results are shown in Fig.3 below.

Total Lipid Content of Selected Isolates using Conventional Extraction Methods.

The results revealed that *Oscllatoria spp*.had the highest percentage of total lipid of 16$\pm$0.5 these results were in harmony with the results of (Christi, 2010)

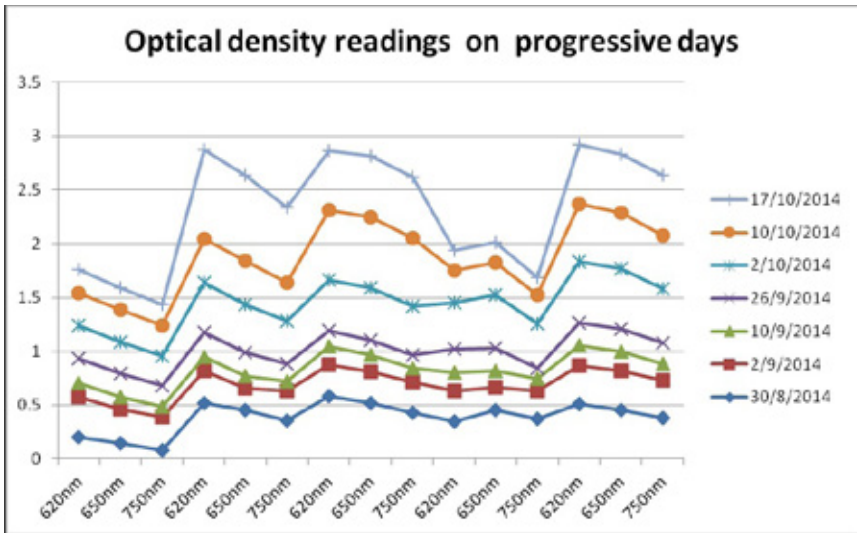

Fig. 3: Graph showing Optical densities on progressive days

who stated that oscillatoria has total lipid content of 16.5%. Two diatoms species were investigated in this study *Nitzschia linearis* and *Navicula cuspidata*. The two species showed relatively low percentage of oil 6.1% and 10.2% for *Nitzschia linearis* and *Navicula cuspidata* respectively. *Nitzschia sp*. was investigated by (Chisti, 2007) and found to have lipid percentage of 45-47%. On the other hand (Sayeda et al., 2014) found that *Nitzschia frustulum* has a total lipid percentage of 13.9%. This percentage near to that presented in this study in table 2

Table 2: Percentages of total oil content in the microalgae studied

Microalgae isolate	Total lipid content %
Oscillatoria sp 1	11± 0.4
Oscillatoria sp.2	16±0.5
Nitzschia lineaus	6 ± 0.1
Navicula cuspulata	10± 0.2

Intracellular lipid droplets of were observed by Nile Red staining under fluorescent microscope with excitation at 450–490-nm and emission at 515-nm (Matsunaga et al., 2009).Neutral lipid or triglycerides appeared as yellow dots, whereas polar lipid and chlorophyll were stained in red colour cells were observed by Nile Red staining under fluorescent microscope with excitation at 450–490-nm and emission at 515-nm.

Nile red staining: Nile red (9-(Diethylamino) -5H benzo [α] phenoxazin- 5-one) staining is specifically used to identify and confirm the intracellular lipid droplets from the biological samples (Greenspan, Mayer, & Fowler, 1985).

The results indicated that not all algal species could be affected by Nile red staining since oil droplets were not clear and the whole cells were stained in red. This was the situation with green isolates, while certain blue green isolates were affected by the dye *where* the yellow stained parts were clear. Referring to *Oscillatoria sp 1* and *Oscillatoria sp 2* the cells showed yellow florescent color under florescent microscope even without adding the dye. So this gives false results when referring to lipid content. Diatoms were stained well with the dye and the oil drops were clear. Since the staining method may not be accurate The florescent method has been applied successfully to the determination of lipids in certain microalgae, but has been unsuccessful in many others, particularly those with thick, rigid cell walls that prevent the penetration of the dye (Held, 2011).Since Nile red method was not accurate in determining lipid content in microalgal cells so lipid content was determined using conventional extraction method using two organic solvents.

CONCLUSION

Results show that physico-chemical parameters play a major role to play in species occurrence. The method of lipid extraction, type of media and level of axenicity of the microalgal species determines the percentage of lipid extracted . *Oscillatoria* species seems to be a hardy species and therefore still survives when subjected to stress. It is therefore recommended for large scale cultivation in photobioreactors for biofuel production. Stress conditions such as nutrient stress results to production of more lipids from the microalgae. Nile red staining is not so accurate therefore should be supplemented by other methods of lipid determnination. The choice of algal strain, the method used for culture and the location of sampling highly determines the amount of lipid produced by microalgae.

RECOMMENDATIONS

The strains used for large-scale algal biofuel production need to be improved through selection and genetic approaches. Breakthroughs and innovations in areas such as increasing the capability of algae to use nutrients efficiently or engineering designs to reduce processing requirements have the potential to greatly improve the energy balance and enhance the overall sustainability of algal biofuels.

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REFERENCES

Abubakar, L. U., & Mutie, A. M. (2012). Characterization of algae oil (oilgae) and its potential as biofuel in Kenya. *Journal of Applied Phytotechnology in Environmental Sanitation*, *1*(4), 147–153.

Alcain, A., Anna, A., & Kushiga, T. (2010). Biodiesel from algae. *Journal of Chemical Engineering*, *Vol.10*, pp.2713-2716.

Al-Handal, A. Y., Al-Assa, S. A., & Al-Mukthar, M. A. (1989). *Journal of Marine Science Center University. Of Basrah*, (4 (1)).

Araujo, G. S., Matos, L. J., Gonçalves, L. R., Fernandes, F. A., & Farias, W. R. (2011). Bioprospecting for oil producing microalgal strains: evaluation of oil and biomass production for ten microalgal strains. *Bioresource Technology*, *102*(8), 5248–5250.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917.

Chen, W., Sommerfeld, M., & Hu, Q. (2011). Microwave-assisted Nile red method for in vivo quantification of neutral lipids in microalgae. *Bioresource Technology*, *102*(1), 135–141.

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, *25*(3), 294–306.

Christi, Y. E. (2010). Feedstocks for second generation biodiesel. *Journal of Microbiology and Biotechnology*, *Vol.20*, pp.3135-3142.

Dragone, G., Fernandes, B. D., Vicente, A. A., & Teixeira, J. A. (2010). Third generation biofuels from microalgae. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, *2*, 1355–1366.

Greenspan, P., Mayer, E. P., & Fowler, S. D. (1985). Nile red: a selective fluorescent stain for intracellular lipid droplets. *The Journal of Cell Biology*, *100*(3), 965–973.

Held, P. (2011). Monitoring Algal Cell Growth Using Absorbance and Fluorescence. *BioTek Application Note.* Retrieved from www.biotek.com.2011.

Matsunaga, T., Matsumoto, M., Maeda, Y., Sugiyama, H., Sato, R., & Tanaka, T. (2009). Characterization of marine microalga, Scenedesmus sp. strain JPCC GA0024 toward biofuel production. *Biotechnology Letters*, *31*(9), 1367–1372.

Pfiester, L. A., Lynch, R., & Wright, T. L. (1980). Species composition and diversity of Phytoplankton in the grand River dam area Oklahoma. *Proceedings of the Oklahoma Academy of. Science*, *Vol 60*, 63–68.

Polat, S., & Aka, A. A. (2007). Total and size fractionated phytoplankton biomass off Karatas, north-eastern Mediterranean coast of Turkey. *Journal of Black Sea/Mediterranean Environment*, *13*(2).

Sayeda, M., Entesar, A., Abo, S., El Enin, R., El Edin, D. S., El Diwani, G., & Gamila, A. (2014). Quantitative and qualitative determination of lipid content in Microalgae for biofuel production. *Journal of Algal Biomass*, *Vol.5(3)*, pp.23-28.

Wilhm, J. L., & Dorris, T. C. (1966). Species diversity of benthic macroinvertebrates in a stream receiving domestic and oil refinery effluentsIn: Islam, S.M. Phytoplankton diversity index with reference to mucalinda serovar Bodh-Gaya,"12th World Lack Conference., Taal, India, . *American Midland Naturalist*, 427–449.

Long Line Seaweed Farming as an Alternative to other Commonly used Methods

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Abstract

To counter the rampant fishing pressure on the over exploited fisheries resources, seaweed farming is gaining momentum as an alternative livelihood among the Kenya coastal communities. There is also a worldwide growing interest in the use of seaweed in food and chemical industries, not forgetting its contribution to the blue economy. In this regard, various methods of seaweed farming like raft method and off bottom method have been used but production is still low. This study aimed at assessing the growth rate and yield of *Kappaphycus alverazzii* (cotonni) and *Eucheuma denticulatum* (spinossum) using the long line floating method. The experiment was performed on 3 plots per species of seaweed with a plant stocking density of 36 seedlings per plot. Measurements of production were done fortnightly. The highest production was realized with *E. denticulatum* during the North East Monsoon (NEM) period (1321.47±93.3 g) with a Daily Growth Rate (DGR) of 6.59% and a yield of 19.9 tonnes/ha. During the South East Moonson (SEM) a weak yield relationship was manifested. *K. alvarezzii* showed a production of 826.27±20g during NEM with a DGR of 5.96% and yield of 14.95 tonnes/ha. During SEM poor daily growth rate of 1.47% and yield of 1.892 tonnes/ha was realized. Based on highest daily growth rate and yield, culture of *E. denticulutum* during the NEM season was optimal.

Key words: Longline method, Kappaphycus alverazzii, Eucheuma denticulatum, seaweed

INTRODUCTION

Globally there is a decline and depletion of fisheries stocks (Naylor et al., 2000), due to overfishing, effects of climate change and pollution. Degradation of these aquatic ecosystems has become a major concern to managers and aquatic resource users (Masese et al., 2013). The dwindling stocks in the capture fisheries have acted as a precursor for rapid growth in aquaculture (Naylor et al., 2000). This is due to the ever mushrooming of human population resulting into more demand for food, employment and poverty eradication. With the world population projected to skyrocket by the year 2050, the demands from the blue economy will be tenfold. However, it is argued that rapid expansion in aquaculture (production of finfish) may solve some of the above challenges man is facing, but it may also aggravate these problems further as far as sustainability of ocean fisheries is concerned. For instance, the farming of crustaceans such as shrimps and finfish (i.e. salmon) farming, may result in habitat destruction, pollution, manifestation of invasive species and to a larger extent depletion of the wild fish stocks via the production of fish meal and fish oil. The growth of aquatic plants (seaweed) may well be a good alternative to fin fish farming in the expansive oceans of the world. This is because of many advantages that it abhors including but not limited to shorter period of time to maturity, easy to culture, poses no

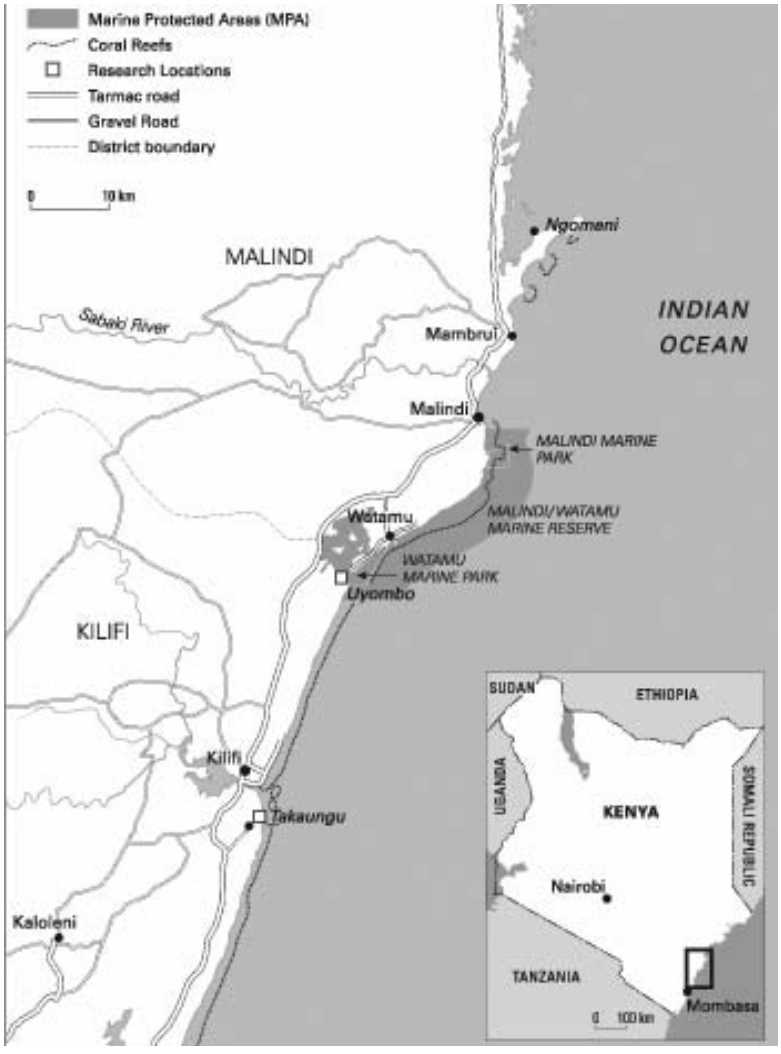

Fig 1. Study area, Takaungu at the northern part of Kenya coastline.

pollution threats given that they mainly utilize the available nutrients, does not require feeding and consequently the cost of production is low. To boost mariculture activities, farmers have embarked on seaweed farming which has been incorporated into many community-based coastal resources management projects and fisheries management initiatives as an alternative livelihood option for fishers in tropical developing countries (Msuya et al., 2014; Msuya, 2006).

Along the Kenya coastline seaweed farming is now being practiced in the south coast at Kibuyuni, Nyumba Sita, Mkwiro, Funzi and Gazi areas. The main species cultured are *Eucheuma denticulatum* (*J. Agardh*) commonly known as 'spinosum' and *Kappaphycus alvarezzi* known as 'cottonni'. Kenya is not endowed with vast well sheltered beaches to venture into sustainable seaweed farming enterprise. To excarbate the challenge even further, it is only *E. denticulatum* that has shown a potential of doing well as compared to *K. alvarezzii*, which is in high demand in the international market hence fetches high prices (Msuya et al., 2014). Other reasons that have been advanced to low production of *K. alvarezzii* include high temperatures due to climate change, invasion by the epiphytes, 'ice ice' condition (a condition associated with the seaweed thallus turning whitish and breaks easily) and high predation by the foraging fishes such as Rabbit fish.

In order to overcome the challenge of suitable sites of seaweed farming in Kenya and more particularly those of farming *K. alvarezzii*, there is need to modify the traditional off-bottom method, employ the use of long line floating method that can make use of deeper waters (Msuya, 2014). Other methods of farming in deep waters include bamboo rafts (Zuberi 2000; Msuya & Salum 2006; Msuya 2011c).

Although farming in deeper waters using the above said alternative methods pose several challenges such as women's participation and a majority can't swim, access to boats and frequent conflicts with fishermen (Msuya 2006b; Msuya et al. 2007b), the methods offer faster growth rates and high production. It has been reported that farming with long line floating method produces 0.35 Kg per unit area compared to off-bottom line method (Msuya et al., 2007a; Msuya 2013a). Higher production has been reported in other countries where deeper water methods have been tried in relation to shallow waters (Hurtado & Agbayani, 2002). The aim of this study, therefore, was to investigate the growth rate and yield of *E. denticulutum* and *K. alvarezzii* grown in longline floating method as an alternative to the commonly used techniques in Kenya.

MATERIALS AND METHODS

Study Area

A preliminary survey of potential study sites along the Northern Kenya coast was done. Takaungu located in Kilifi County at latitude of S 03° 43' 4.09" and a longitude of E 039° 51' 41.60" proved the best. The site was selected because it is sheltered from wave action, accessible and represent a range of environmental conditions in Kenya. The coastal belt of Kenya experiences a tropical monsoon climate dominated by two seasons, the Southeast Monsoon (SEM) locally referred as *Kusi* prevailing from May to October and the Northeast Monsoon (NEM) locally referred as *Kaskazi* December to March. The study period fell under the two seasons and therefore growth comparisons was done for SEM and NEM. The two seasons are characterized by distinct differences in physical and chemical conditions of the coastal waters (Church & Obura, 2004).

Long Line Seaweed Farming Method

A plot measuring 5 m X 1.5 m was made with 3 lines made up of 12 seedlings each. Therefore there was a plant stocking density of 36 seedlings per plot. To make the plot, a 3 mm thick polypropylene rope of 5 m long was stretched between anchors and floaters. 15 pieces of raffia (polypropylene strings) were attached to the 3mm rope. 3 lines of the same size were made and tied to bamboo (floaters) at both ends.

Luxuriant and strong branches of seaweeds sourced from Kibuyuni were used as seed for planting, where cutting was done using a sharp knife, cleaned of silt and the associated plants and animals. The two species of seedlings *E. denticulatum* and *K. alvarezzi* were weighed differently using a laboratory spring balance (single/dual scale capacity) to determine the initial weight before planting. The initial weight ranged between (35-75 g). The seedlings were then tied on the ropes at their strongest points.

A clear site was chosen with clear water, tidal range, moderate water current and protected from large waves and strong winds, water temperature between 27°C to 30°C, salinity ranged from 30 to 35 ppm, water depth of 1-4 m at the lowest tide (Msuya, 2006a). Anchors were placed into the substratum; the seaweed lines were then carried to the growing site and tied to the floaters. The experimental set up was done in triplicate for both species. A total of 6 plots were made and 3 groups from the Takaungu community assigned 2 plots each to take care of. Monitoring was scheduled to take place after every 2 weeks during low tide for 45 days. Growth rate was evaluated during the NEM and SEM seasons of the year.

Data Analysis

Data on growth rate was presented as means (±SEM). Productivity as specific percent per day was based on the increase in biomass per unit time and calculated as daily growth rate (DGR)% d^{-1} and determined from the following formula: (Wakibia *et al,* 2006; Evans, 1972)

$$RGR = [(Wt/Wo)1/t - 1] \times 100$$

Where, *Wo* = average cutting wet weight at start,

Wt = average cutting wet weight at time t and

t = time intervals (days).

Plant yield (Y) expressed as estimated kg/m^2 was determined using a formula modified by Hurtado *et al.* (2001):

$Y=(W_t/W_o)/A_t$

Where W_t and W_o are as in equation 1

A_t= total area of the plot

The results from the plots were extrapolated to determine what one hectare of seaweed plot could produce in tonnes. The data was plotted and the growth rates calculated in Excel for Windows 2011. The Kolmogorov-Smirnoff test was used to test for normal distribution of the data. ANOVA was used to test for differences in mean growth rates and yield between the replicates of each method and all inferences were accepted at α=0.05.

RESULTS

Growth Rate and Yield of *E. denticulatum*

E. denticulatum performed very well under the long line floating method in both seasons of the study period (SEM and NEM). Its growth rate during NEM is as illustrated in Fig.s 2 and 3.

By the time of maturity/harvest (i.e. 45th day), *E. denticulatum* in plots 1, 2 and 3 attained 830.6167±93.703g, 1321.417±93.310g and 1080.513±90.956g of weight respectively during the NEM season. In all the 3 plots, growth rate of *E. denticulatum* increased steadily with time in spite of small variation among the plots (Fig. 2).

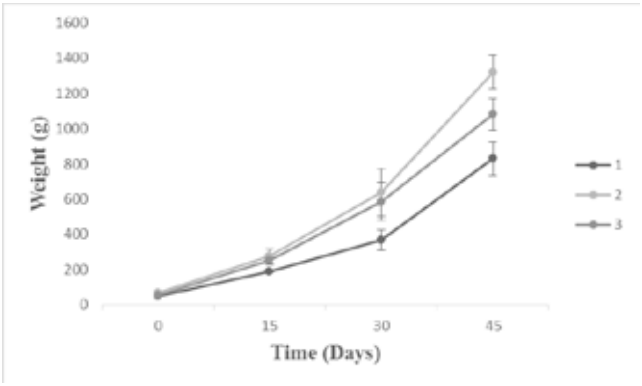

Fig.2. Growth rate of Eucheuma denticulatum in the three plots during NEM season

Generally the growth rate of *E. denticulatum* during the NEM season was 1077.516±84.651g by the 45th day of planting (Fig.3). Seaweed of this species grown in this area could attain a maximum weight of 532.62±67.17g by the 30th day of planting while it could attain a weight of 251±22g by 15th day of planting.

The Daily Growth Rate (%) of *E. denticulatum* during the NEM season was found to be 6.59% and that 19.9 tonnes could be produced from a one hectare farm. DGR%d^{-1} did not differ significantly between the plots during the NEM season of the study period. The yield also showed no significant difference between the plots. However, the yield showed a weak relationship

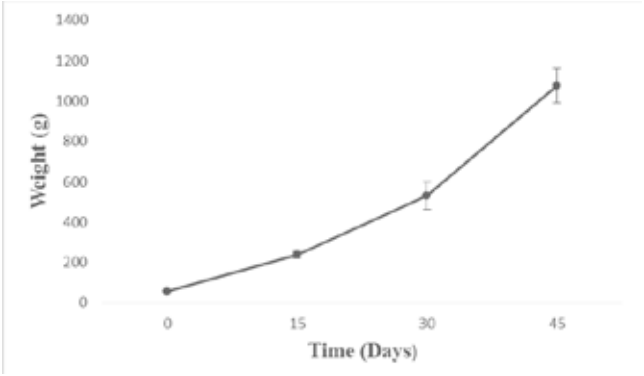

Fig. 3. General Growth rate of Eucheuma denticulatum in the study area during NEM season

(p=0.07) between the plots.

The performance of *E. denticulatum* during SEM is as shown in Fig.s 4 and 5. During the SEM season of the study period, *E. denticulatum* attained a maximum weight of 982.277±81.771g. However, during the same period the seaweed increased in weight steadily (Fig. 5). DGR % d^{-1} did not differ significantly between the plots during the SEM season of the study period. However, the yield showed a weak relationship (p=0.07) between the plots. There was a weak relationship (p<0.05) observed between the growth rates of the *E. denticulatum* at day 45 during the SEM season.

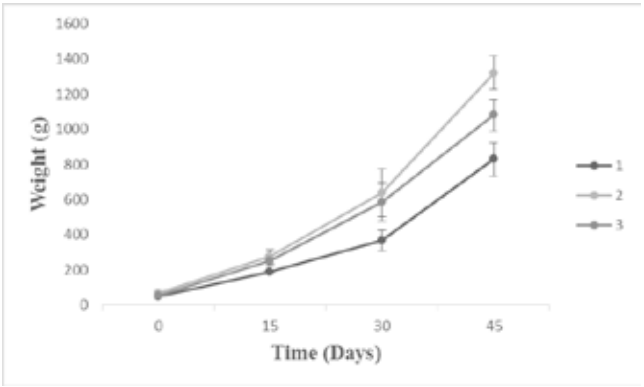

Fig. 4. Growth rate of Eucheuma denticulatum in the three plots of the study area during the SEM season

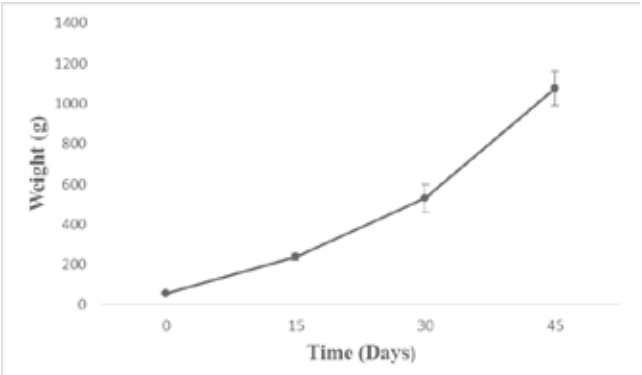

Fig. 5. The general growth rate of Eucheuma denticulatum in the study area during the SEM season

Growth Rate and Yield of K. alverazzii

The performance of *K. alverazzii* during the NEM season was

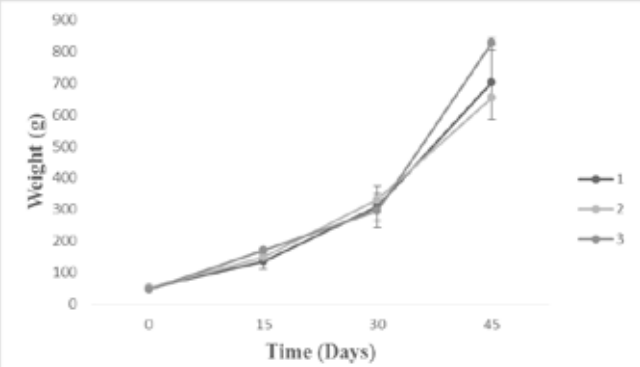

Fig. 6. Growth rate of Kappaphycus alverazzii in the three plots during NEM season

fairly good (Fig. 6). Plot 3 recorded the highest growth rate (826.27±20) followed by plot 1 (703.52±116.42g) and then lastly plot 2 with an average weight of 654.85± 1.26g (Fig. 6). The growth rates of *K. alverazzii* did not differ significantly between the plots during the NEM season.

The general performance of *K.alverazzii* in the study area during NEM season was as indicated in Fig. 7. This seaweed species attained a maximum weight of 728.21±42.59g at maturity (45 days). For the first 30 days the growth rate was somehow slow (311.18±22.04g) as compared to the last 15 days to maturity when its growth was accelerated. The DGR% of *K. alverazzii* during the NEM season was found to be 5.96% and the total yield during this time when extrapolated was found to be 14.95 tonnes per hectare. DGR%d^{-1} did not differ significantly (p=0.94) between the plots during the NEM season of the study period. However, the yield showed a weak relationship (p=0.063) between the plots.

During SEM season, *K. alverazzii* displayed a very poor growth

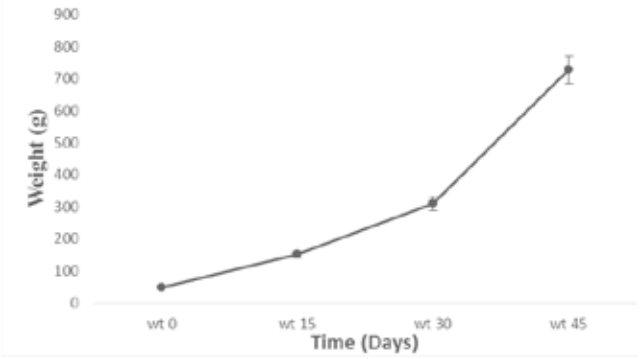

Fig. 7. General Growth rate of Kappaphycus alverazzii in the study area during the NEM season

rate (see Figs. 8 and 9). Although all the three plots performed dismally during the SEM season of the study period, plot 3 recorded the highest weight of (103.74±8.2g) followed by plot 1 (87.38±4.21g) and then plot 2 which recorded 87.323±9.0g (Fig. 9). *K. alverazzii* managed to record a maximum weight of 92.81±3.97g at maturity during the SEM season of the study period. During this season (SEM) *K. alverazzii* recorded

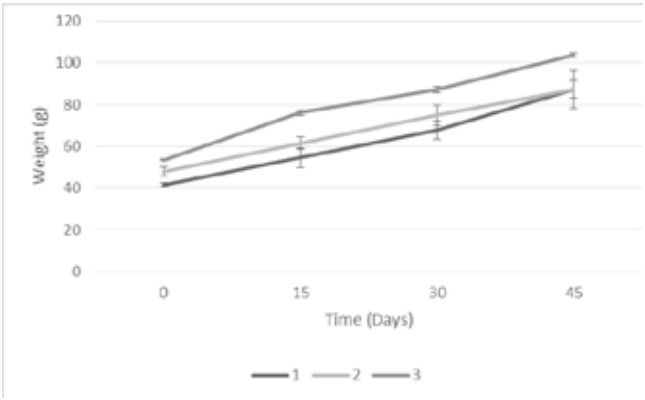

Fig. 8. Growth rate of Kappaphycus alverazzii in the three plots during SEM season

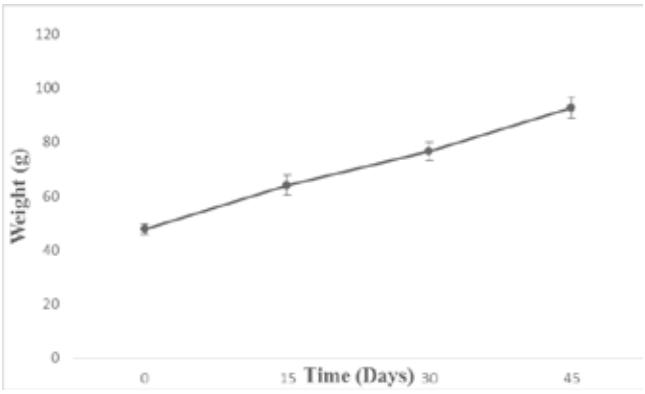

Fig. 9. General Growth rate of Kappaphycus alverazzii in the study area during the SEM season

DGR%d^{-1} of 1.47% whereas the yield was found to be 1.892 tonnes per hectare. There was no significant difference observed on the daily growth rates between the plots and yield of *K. alverazzii* during the SEM season.

When comparing the growth rates of the two seaweed species, it was found that *Eucheuma denticulatum* performed well as compared to *K. alverazzii* in all the seasons during the study period. Although *K. alverazzii* tried to perform well during the NEM season, its performance did not however out do that one of *E. denticulatum* in both seasons (i.e. SEM and NEM).

DISCUSSION

During the monitoring exercise weather conditions were generally variable with moments of sunny and rainy periods. Generally there was marked improvement in the growth rate of both *E. denticulatum* (Spinosum) and *K. alverazzii* (Cottonii) species in all the plots during the NEM season of the study period. This could be explained by the calm weather patterns during this time hence sedimentation was low enabling the plants to photosynthesis food because of enough penetration of light into the water system. The calm weather may have contributed to less velocity of the water thereby the seaweed propagules remaining intact i.e. they did not break away hence a bumper harvest/growth rate.

However, poor growth rate of *K. alverazzii* was evident as compared to *E. denticulatum* during both seasons. The morphological makeup of *K. alverazzii* thallus is thicker as compared to that

one of *E. denticulatum* such that when it has attained a reasonable growth it becomes heavier thereby displaying a brittle like characteristic whenever there is little water current. Therefore chances are that it may have broken time and again thereby swept away. Another reason that has been advanced to this effect is that *K. alverazzii* is a good source of food for rabbit fish hence because of the small farms the grazers overwhelmed its existence accounting for poor growth rates and yields (Msuya, 2006a,b). Doty (1973) blames grazing on plants by sea urchins and siganid fishes as the cause of failure for the plantings on bottoms. Also Parker (1974) has observed non-branched appearance of native *Eucheuma* strains growing in areas densely populated by sea urchins in the Philippines. Ask (1999) observed several fish taxa eating tips of plants. This is a serious problem since the tips are the growth area of the plant, and it can take weeks for a new tip to grow. Other fish attach plants voraciously and remove the entire cortical layer of the plant. Msuya *et al.* (1996) reports that grazing on seaweeds by box fish, rabbit fish and sea urchins reduce the growth rate.

In order to overcome the herbivory nature of the fishes on this species of seaweed, it is recommended that big farms be done (Wakibia et al., 2006). Rapid grazing of the seaweed causes a disease called ice-ice that gets an opportunity to thrive well on the cut ends of the thallus, thus complicating the situation even further. This makes the seaweed to easily die hence reducing the yield of the farm.

During SEM, the performance of *E. denticulatum* was not as good as during the NEM season although in both seasons it seemed to do well. This could be explained by the fact that during SEM the velocity of the water in the sea is very fast, interfering with the seaweed thallus especially in situations where the tie ties were too tight: they could easily break away leaving behind smaller attachments. Also because of fast movement of the water, turbidity becomes high as indicated by the level of conductivity during the study period. This situation hampers the development of the plant because of low rate of photosynthesis. Msuya (2006a) argues that light is of paramount essence when growing seaweeds. She also portends that in order for the weed to do well, a well sheltered site is a must. Such a site prevents the strong currents from the open sea from interfering with the tied propagules of the seaweed. In general the performance of *E. denticulatum* was good going by the recommended net weight that should be attained by week 6 of planting (at least 650g) (Wakibia, *et al*., 2006). Some of the other factors that led to good growth rates of *E. denticulatum* included the water quality parameters although they were not measured.

During SEM *K. alverazzii* performed dismally most probably due to invasion by epiphytes that may have increased competition for food. Epiphytism is a major worldwide problem in the cultivation of *K. alverazzii*, which severely reduces the productivity and cost efficiency in open water cultivation. Large algae may overgrow the farmed plants and thereby compete with the farmed plants for sunlight and nutrients (Buschmann & Gomez, 1993). These are *Enteromorpha, Ulva, Chaetomorpha* (Chlorophyta), *Hypnea* (Rhodophyta) and *Hydroclathrus* (Phaeophyta). They reduce the inorganic carbon uptake of *E. alverazzi* due to elevated pH (Mtolera *et al.,* 1995a, b). Because farmers didn't remove the epiphytes early enough given that they had to wait until we go to the site after two weeks, this could have compounded the problem. The farmer has to remove the epiphytes and competitors physically. If removed as soon as it settles in the farm it may be possible to decrease the problem. Azanza *et al*., (1996; 1992) suggests that these algae should be taken to the shore and that they might be used as fertilizer on land, sedimentation and herbivory by the fishes.

CONCLUSION

In conclusion, long line floating method performed well in the study area. It was also observed that *E.denticulatum* did well as compared to *K.alverazzii*. Therefore, seaweed farming can do well in this area while growing spinosum using long line floating method. The study recommends up scaling of *E.denticulatum* species under longline floating method. However, should good progress be anticipated, then the community needs a technical team that will guide them on a regular basis.

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REFERENCES

Ask, E. I. (1999). Cottonii and spinosum cultivation handbook. *FMC Food Ingredients Division, Philadelphia*, *52*.

Azanza-Corrales, R., Aliaza, T. T., & Montano, N. E. (1996). Recruitment of Eucheuma and Kappaphycus on a farm in Tawi-Tawi, Philippines. *Hydrobiologia*, *326*(1), 235–244.

Azanza-Corrales, R., Mamauag, S. S., Alfiler, E., & Orolfo, M. J. (1992). Reproduction in Eucheuma denticulatum (Burman) Collins and Hervey and Kappaphycus alvarezii (Doty) Doty farmed in Danajon Reef, Philippines. *Aquaculture*, *103*(1), 29–34.

Buschmann, A. H., & Gómez, P. (1993). Interaction mechanisms between Gracilaria chilensis (Rhodophyta) and epiphytes. *Hydrobiologia*, *260*(1), 345–351.

Church, J., & Obura, D. O. (2004). Management recommenda-

tions for the Kiunga Marine National Reserve, based on Coral reef and fisheries catch surveys, 1998–2003. *CORDIO/WWF KMNR, Lamu, Kenya*, 1–57.

Doty, M. S. (1973). Farming the red seaweed, Eucheuma, for carrageenans. *Micronesica*.

Evans, G. C. (1972). *The quantitative analysis of plant growth* (Vol. 1). Univ of California Press.

Hurtado, A. Q., & Agbayani, R. F. (2002). Deep-sea farming of Kappaphycus using the multiple raft, long-line method. *Botanica Marina*, *45*(5), 438–444.

Hurtado, A. Q., Agbayani, R. F., Sanares, R., & de Castro-Mallare, M. T. R. (2001). The seasonality and economic feasibility of cultivating Kappaphycus alvarezii in Panagatan Cays, Caluya, Antique, Philippines. *Aquaculture*, *199*(3–4), 295–310.

Masese, F. O., Muchiri, M., & Raburu, P. O. (2009). Macroinvertebrate assemblages as biological indicators of water quality in the Moiben River, Kenya. *African Journal of Aquatic Science*, *34*(1), 15–26.

Msuya, F. E. (2011). Experimental farming of the seaweed Kappaphycus in floating rafts in Pemba Island, Zanzibar, Tanzania BIRR-MACEMP project phase II. *Consultancy Report Submitted to BIRR Seaweed Company*.

Msuya, F. E., Mmochi, A. J., & Muhondo, C. A. (2007). Zoning of the Mlingotini Bay in Bagamoyo, Tanzania to solve conflicts between seaweed farmers and other users of the Bay. *2nd Annual Agricultural Research Review Workshop*.

Msuya, F. E., & Salum, D. (2006). The Effect of cultivation duration, seasonality, and nutrient concentration on the growth rate and biomass yield of the seaweeds Kappaphycus alvarezii and Eucheuma denticulatum in Zanzibar, Tanzania. *Western Indian Ocean Marine Sciences Association (WIOMSA), Zanzibar (Tanzania)*.

Msuya, Flower E. (2006a). Seaweed farming as a potential cluster.

Msuya, Flower E. (2006b). The seaweed cluster initiative in Zanzibar, Tanzania.

Msuya, Flower E., Buriyo, A., Omar, I., Pascal, B., Narrain, K., Ravina, J. J., … Wakibia, J. G. (2014). Cultivation and utilisation of red seaweeds in the Western Indian Ocean (WIO) Region. *Journal of Applied Phycology*, *26*(2), 699–705.

Msuya, Flower E., Mmochi, A. J., Sullivan, K. A., Crawford, B., Shalli, M. S., & Tobey, J. (2007). A comparative economic analysis of two seaweed farming methods in Tanzania.

Mtolera, M., Collen, J., & Pedersen, J. (1995). Photoinhibition of photosynthesis in thwe tropixcal marinew maxcroalgawe Eucheuma denticulatum (Rhodophyta). The role of ultraviolet-B radiation during spring tides. In Photosynthesis, growth and light-induced stress responses in the red alga Eucheuma denticulatum. *Phd Thesis of M. Mtolera., Faculty of Science and Technology, Uppsala University*.

Mtolera, M. S., Collén, J., Pedersén, M., & Semesi, A. K. (1995). Destructive hydrogen peroxide production in Eucheuma denticulatum (Rhodophyta) during stress caused by elevated pH, high light intensities and competition with other species. *European Journal of Phycology*, *30*(4), 289–297.

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C., Clay, J., … Troell, M. (2000). Effect of aquaculture on world fish supplies. *Nature*, *405*(6790), 1017.

Parker, H. S. (1974). The culture of the red algal genus Eucheuma in the Philippines. *Aquaculture*, *3*(4), 425–439.

Wakibia, J. G., Bolton, J. J., Keats, D. W., & Raitt, L. M. (2006). Factors influencing the growth rates of three commercial eucheumoids at coastal sites in southern Kenya. *Journal of Applied Phycology*, *18*(3–5), 565–573.

Zuberi, L. (2000). The Final Report on Seaweed Farming Raft Method of Cultivation, Held at Moa, Kijiru Villages of Tanga Region. *Tanga Coastal Zone Conservation and Development Programme. 3p*.

Review of Coral Classification Status:
A Case Study of Kiunga Marine National Reserve, North Coast Kenya

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Abstract

Coral classification is a dynamic science which requires regular updates on the species name changes, distribution, threats of extinction and the methods used to detect these changes. Use of a single method of classification either morphological or genetic is not sufficient to confirm the changes. A review of Coral classification for the Kiunga Marine National Reserve (KMNR) in north coast Kenya was conducted during October, 2014 and July 2015 to ascertain the current status and assess the coverage on phylogenetic literature; IUCN Red List status and reference to threats facing the species which are local or elsewhere. Results from this study showed that Corals from Kiunga Marine National Park were well covered in all aspects of phylogenetic literature at 89.4% making it possible to adequately group them to their IUCN Red list categories. However, the analysis detected a group of species which had not been assigned into any status on the IUCN Red list classification, representing 8.6%. With data and information on such omissions, the species could be faced with several threats hence the need to conduct further studies and analysis focusing on acquiring comprehensive data and information for clear establishment of updated status of the taxonomy of Corals so as to correctly label them in the groups.

Key words: Morphological Classification, Genetic Classification, IUCN Red list Categories, Phylogenetic, Coral Species.

INTRODUCTION

The process of Coral classification revision, addition to distribution ranges, discovery of new species and other type of information will continue indefinitely (Veron, 2000). In the current century, global Coral species are classified on the bases of morphological and genetic analysis, (Budd *et al*., 2010 & 2014; Veron, 2000). Morphological classification of Coral species is classification which uses the physical, readily observable features such as calcification forms, shape, size, color and positioning of centers in Corals (Budd *et al*., 2010; Veron, 2000). In genetic classification, internal structures such as nuclear markers are investigated and the results allows Corals to be classified in terms of their spawning patterns, rates at which they lose their skeleton due to stress, level of extinction, susceptibility to diseases or sometime their distribution pattern (Fukami *et al*., 2008). This study review these two modes of classification to show how they are commonly being used as reference for comprehensive understanding of the Scleractinian species, (Budd *et.al.,* 2010) and propose for the same to be applied in the classification of Kenya's Coral reefs.

In Kenya, Coral classification is mainly based on morphological analysis (KWS, 2001), (WWF, 1996; Obura, 1998 & 2006; Veron, 2000), and this method limits the number of analysis that could be done on identified Coral species. To understand and increase the efficiency to the full range of classification variations of all Coral species in Kenya would involve first review of phylogenetic literature. This will increase our understanding of reclassification (genetic and morphologic), distribution or abundance of threatened species and enable prioritization of future projects on data deficient species. In this review, focus is on Kiunga Marine National Reserve (KMNR) for its mixed East Africa and Gulf of Aden Coral species and which are adapted to colder ocean upwelling conditions. This provides a curiosity to understand their genetic interaction and any changes in their biogeography.

General Objective

To review the Corals classification status; groups, identification and naming.

Specific Objectives:

- To assess morphological and genetic classification literature of the Coral species.
- To assess the dominant families of Coral species studied.
- To assess the dominant red-list categories of the studied Corals species.

Hypothesis

There is a complete cover in Coral classification status in Kiunga Marine National Reserve.

Justification

Documentation of Coral taxonomy in Kenya is always getting some new inputs (Obura, 2012; Arrigoni 2012) and this review of Coral classification status will combine existing knowledge of Corals classification through morphology with genetic classification, to make it more useful in assessing Corals risk factors.

Literature Review

Coral reef ecosystems significantly contribute to the health and wellbeing of the coastal environments. Scientist 2012, estimated 1 to 3 million species depended on Corals (Kumaraguru, et al., 2003). Globally, approximately 500 million people depend on Coral reef ecosystem for subsistence of which about 15 to 20 million people (McClanahan, et.al, 2008, Wanyonyi et al., 2008), are located within the Western Indian Ocean (WIO), (Secretariat of the Convention on Biological Diversity, 2010). Some of the different direct benefits from Corals include food in form of fish, invertebrates and algae, none food goods which include building and pharmaceuticals materials, service benefits such as recreation and tourism industry and physical barrier for coastal protection (Spalding et al., 2001; Cesar, 2002). Even with these immense uses, the Coral reef still faces the challenge of negligence especially on the part of classification and extinction risks unlike other terrestrial ecosystem (Budd et al., 2012).

21st century Coral conservation efforts begins with recognition of common biogeographic distribution patterns (Obura, 2012), that may lead to identification of priority classification groups. Kitano (2014), observed that using revised data on Coral classification to carry out different analysis such as risk of extinction can aid in compiling conservation strategy plans to counter threats facing Corals of the world. Huang (2012), noted that data deficient species require to be assessed regularly so as to update the necessary information for all Corals. This appreciation could start with local understanding of Coral species, their current classification and conservation status.

Decisions on design of Community Conservation Areas (CCAs), Beach Management Units (BMUs), and Marine Protected Areas (MPAs), are based on the distribution of species with the highest national or international values, risks, species of concern and high biodiversity (Huang *et al.*, 2012). Today, biogeographic dataset and abundance is important especially in assessment of Corals species extinction risk and can be used to classify Coral species in the IUCN Red List of Endangered Species (Obura, 2012).

Corals of the Kenyan Coast

KMNR Corals of Kenya's north coast form part of the East African fringing reef System (Obura & Church, 2004), and have been observed to be smaller in terms of coverage, size and diversity (Obura, 2012). This area has cooler and nutrient rich ocean condition resulting from the Somali current upwelling and has been suggested to cause the low number of Corals in the area (Obura & Church, 2004).

These Corals form important habitats linked to form a fringing reef system which support local livelihood and other natural services. To conserve these habitat, Kenya has put up some marine areas under protection, for example, Kiunga Marine National Reserve, Mombasa Marine National Park and Reserve, Malindi Marine National Park and Reserve. For years, these conservation areas have been used for monitoring the country's Coral diversity. From the monitoring expeditions, about a dozen Coral species have continuously been identified (Obura, 2012).

There are around 200 recorded species of hard Corals in Kenyan Coral reefs (Obura *et al*, 2008). From this record, the dominant species include the massive reef-building Coral Porites spp., *Acropora, Pocillopora, Favia, Favites* (Hamilton & Brakel, 1984). By using physical factors such as tidal current, wave exposure and depth, the Coral reef in KMNR has been divided into; high-energy fore-reef slopes; moderate-energy slopes and back-reef patches; and sheltered back-reef and island-channel patches, (Church & Obura, 2004b; Obura & Church, 2004).

With a well updated database of Coral species, their distributions and any new additions, will help in further understanding their place in future of changing marine environment. Huang (2012), observed that Coral analyses that dealt with phylogenetic clustering of susceptibilities, resistance and resilience to various risk factors drew much of the data from precisely described specific species data.

Reclassification of KMNR Corals

Obura (2012), investigated the presence or absence of Coral species in the WIO region in an effort to close the gap on coarse scale data on biogeography which can be considered for conservation planning in the ecoregion. One of the findings was that Kenyan Corals have seen some systematic changes in respect to their distribution such as Corals classified as East Coast Corals and Northern Mozambique Channel Corals. Reviewing more status of these Corals would add more details which would be considered when confirming these changes. Currently, Coral genetic analysis has become important in understanding species susceptibility to threats, distribution and diversity. Rigorous review of this data to the simplest way such the most common genetically analysed taxa, will give priority additional information when working around species distribution, diversity or conservation decisions.

Worldwide, Corals are majorly affected by the extreme surface warming of the sea resulting to ocean acidification. The lowering of ocean pH (ocean acidification) subjects Coral reefs to severe stresses making the habitats become prone to intensified bleaching, diseases and reduced calcification rates for the exoskeleton organisms (Huang, *et al.*, 2012). These natural factors together with anthropogenic impacts such as pollution increases the rate of Coral reefs shifts toward microalgae domination hence reducing their survival. Accurately grouping these Coral species according to level of susceptibility to any of the threats can give Coral ecologist insight on conservation prioritization.

El Nino effects on Coral diversity and distribution in East Africa as reported by David *et.al* (2000), was observed to cause specific species pattern of bleaching. The most affected species were

observed to occur in family Acroporidae and were indicated to be or close to 100% bleached and dead (Obura, *et al.,* 2000). A key recommendation from this study was management issue which would focus on using scientific information, genetic analysis and monitoring in the management decision making.

At present, Kenya Coral monitoring is based on Coral cover, disease infected, bleached and recovery status (Secretariat of the Convention on Biological Diversity, 2010). To further understand the preference of threats to Corals worldwide, recent studies are focusing on specific Coral indicators on resilience, recovery and adaptability to changing environmental conditions (Obura, *et.al*, 2008). It has been observed that a great deal of Indian Ocean, Kiunga Marine National Reserve, Coral species have not been exhaustively analyzed hence their taxonomy placement is still questionable (Arrigoni, *et al*., 2012).

Traditional Coral taxonomy, which is still being used in Kenya, is based on the examination of the Coral skeleton at a macroscopic level using binocular microscope (Budd, *et.al*, 2012). To the rest of the world, this traditional taxonomy has, to some extent, been criticized and has lend to adaption of new mode of classification through the use of molecular analysis. The molecular method gives finer details in Corals enabling precise taxonomy revision. Its use in Coral classification extends beyond the recognition of distinct taxa that are at risk. For example the natural hierarchical status of phylogenies may sometime lead to misconception, where random losses of species are seen not to disturb a group in classification whereas concentration of threatened species in another particular group can cause pronounced systematic changes (Huang *et al*., 2012). Recent studies, (Budd *et al*., 2012; Fukami *et al*., 2008, Budd & Stolarski 2009 & 2011) have vested in molecular analysis as basis for new revised Coral classification.

Even with the molecular analysis, Kitano (2014), observed that a conflict sometimes occurs between the molecular phylogenetic analyses of Scleractinian Corals and traditional morphology-based taxonomy. Therefore, having regular reviews on the Coral classifications status; abundance, distribution, risk categories and any new additional data or knowledge will help Coral taxonomist, biologist and conservationist to keep up with any change on these species.

MATERIAL AND METHODS

Study Area

Kiunga Marine National Reserve (KMNR), Fig. 1, is to the north most part of the Kenya coastline, coordinate (40°07' E, 2°00' S). It is at the confluence of two major ocean currents; the north-flowing East African Coastal Current and the south-flowing Somali Current which creates nutrient rich upwelling (Church & Obura, 2006). The Coral reefs found within KMNR are comprised of mainly patch reefs and fringing reef. These reefs are home to over 50 genera and 150 identified Coral species, (Obura, *et al*., 2007). The reserve was established in 1979 with aim to safeguard the biodiversity and integrity of physical and ecological processes of KMNR, for the health, welfare, enjoyment and inspiration of present and future generations (WWF, 1996). The area is currently under investigation to further projects on biodiversity assessment (David 2015 unpublished).

Data Collection

This paper uses a species list of Corals from the Kiunga Marine Reserve collected by David Obura from 1999 to 2008. Given the significant changes in Coral taxonomy and systematics explained earlier, we then assessed to what extent each of these species were considered in the taxonomic literature (Table 1) and their IUCN Red List status

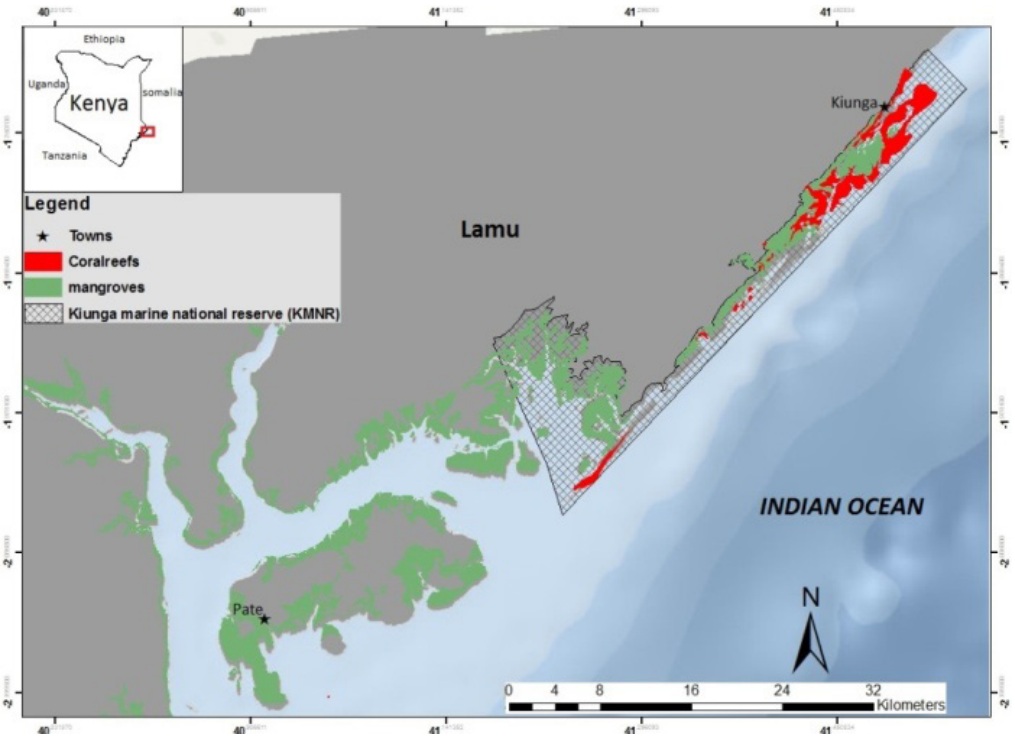

Fig. 1. Map of Kiunga Marine National Reserves Coral reef survey areas. Source (CORDIO East Africa Database 2015).

Table 2. Phylogenetic literature publications.

Authority	Publication title
Arrigoni-2012*	Molecular Phylogeny of the Robust Clade
Budd-2010*	Rethinking the Phylogeny of Scleractinian Corals
Budd-2011	Corallite Wall and Septal Microstructure in Scleractinian Reef Corals
Budd-2012*	Taxonomic Classification of the Reef Coral Family Mussidae
Faure-2008	List of Scleractinian Coral Species cited from the Mascarene Archipelago
Fukami-2008*	Mitochondrial and Nuclear Genes Corals study
Fukami-2008	Short review molecular phylogenetic analyses of Reef Corals
Huang-2011*	Cleaning up the 'Bigmessidae' Molecular phylogeny of Scleractinian Corals from Faviidae Merulinidae Pectiniidae and Trachyphylliidae
Huang-2012*	Threatened Reef Corals of the World
Huang-2014	Towards a Phylogenetic Classification of Reef Corals the Indo-Pacific Genera Merulina Goniastrea and Scapophyllia
Kitahara-2010	A Comprehensive Phylogenetic Analysis of the Scleractinia
Kitano-2014	A Phylogeny of the Family Poritidae
Obura-2012	The Diversity and Biogeography of Western Indian Ocean Reef
van Woesik-2003	Scleractinian Taxonomy
Wallace-2005	Biodiversity of the Indian Ocean from the perspective of Staghorn Corals

Data Analysis

In this study data was analyzed in Microsoft Excel for relationship of the Coral classification publications, genetically studied families and Red-List extinction factors.

RESULTS

The study reviewed families of hard Coral and they genera recorded in KMNR, table 3. In the phylogenetic literature coverage of KMNR species, six peer-reviewed studies looking at the new phylogenetic of Corals were reviewed, stared in table 1. The species were grouped into five categories: included in all (6), most (4-5), few (2-3), just one (1) or not included in the studies in Fig. 2

Table 3. IUCN Red List categories.

Red-list status	
XX	Not evaluated
DD	Data Deficient
LC	Least concern
NT	Near threatened
VU	Vulnerable
EN	Endangered
CR	Critically endangered
EW	Extinct in the wild
EX	Extinct

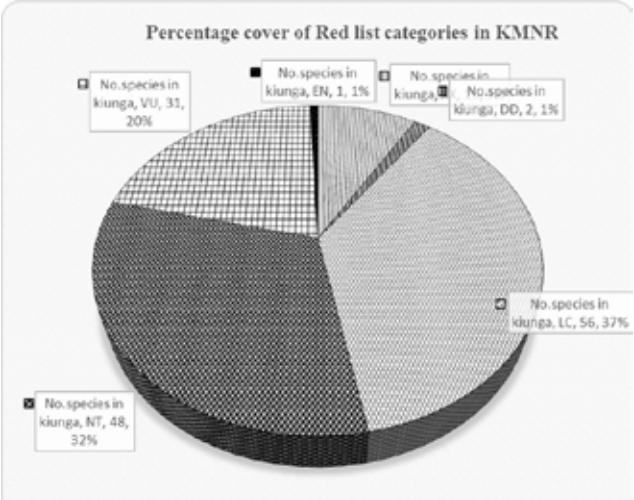

Fig. 2. Pie chart with percentage cover of Red List categories in KMNR.

Table 4. Hard Coral families and the number of genera and species recorded in the KMNR.

Family	No. of genera	No. species
Acroporidae	**4**	24
Agariciidae	**3**	9
Coscinaraeidae	**3**	7
Dendrophylliidae	**1**	5
Faviidae	**11**	53
Fungiidae	**3**	7
Hydrozoa	**1**	1
Meandrinidae	**1**	1
Merulinidae	**2**	2
Mussidae	**5**	11
Oculinidae	**1**	1
Pectiniidae	**3**	3
Pocilloporidae	**2**	7
Poritidae	**3**	16
Siderastreidae	**2**	4
	45	151

A combination of these data results gives, table 1 and Fig. 3, number of species recorded in each family and their phylogenetic literature coverage.

Further, Fig. 4 shows the extent of phylogenetic cover in select dominant families while table 5 outlines how these families species are categorised in IUCN Red List.

Fig. 5, 6,& 7 matches the relation of the IUCN Red List and phylogenetic studies giving a picture of which Coral family has received much attention and which need to be revisited.

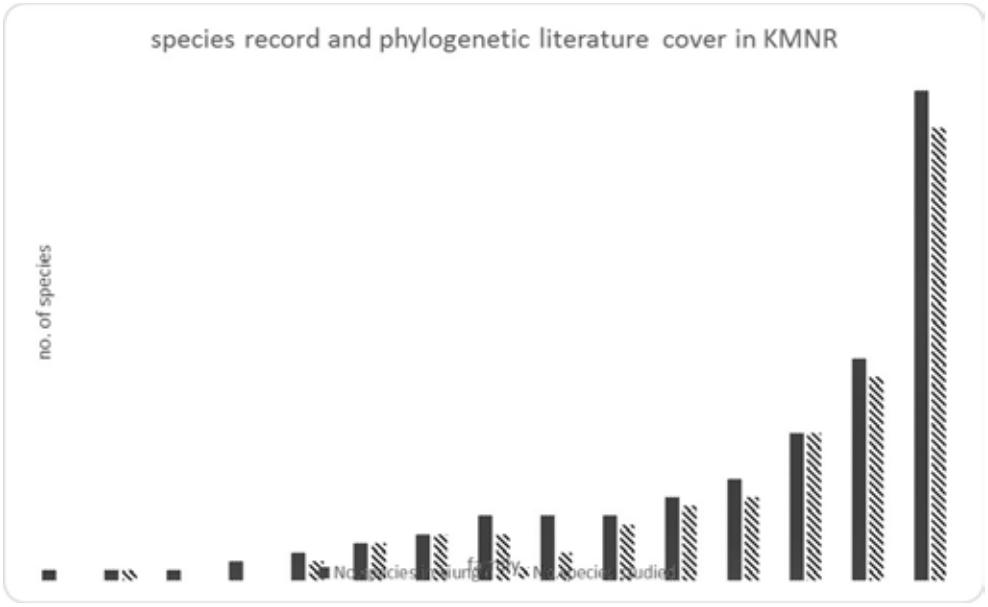

Fig. 3. Coral family graph with number of species recorded in KMNR and their phylogenetic literature coverage.

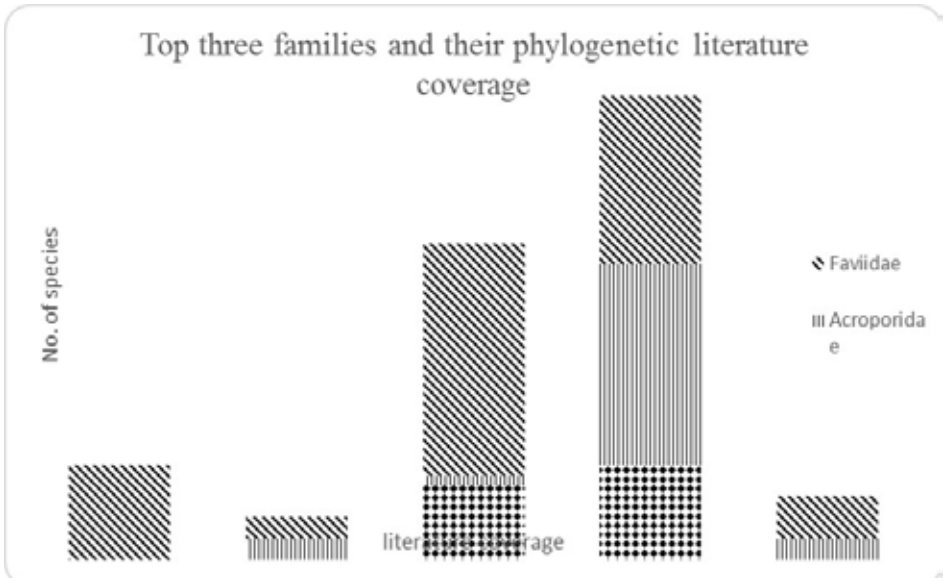

Fig. 4. Phylogenetic literature coverage graph of top three families.

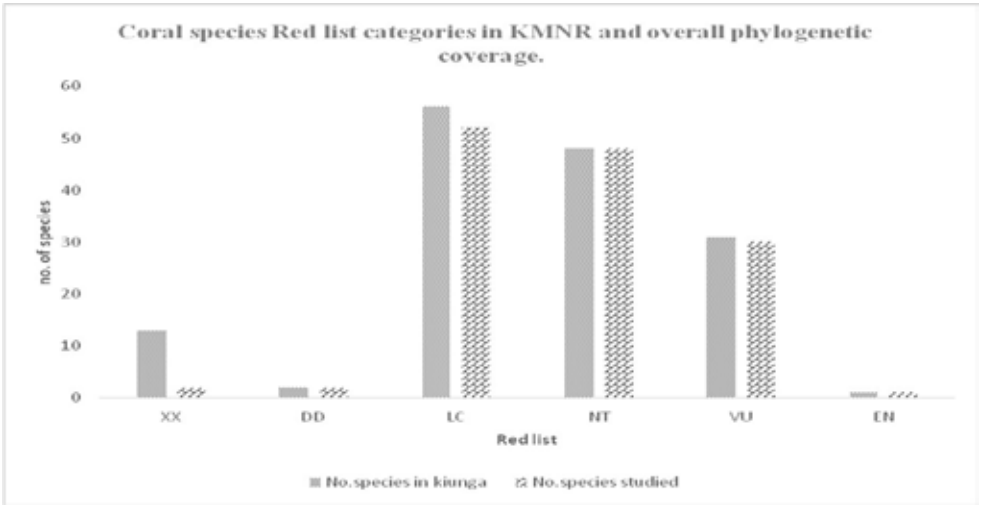

Fig. 5. Coral species Red list categories graph showing phylogenetic literature coverage

Table 5. Number of species recorded in each family and their phylogenetic literature coverage.

Family	No.species in kiunga	No.species studied	Included in all (6)	Most (4-5)	Few (2-3)	Just (1)	Not included
Hydrozoa	1						1
Meandrinidae	1	1		1			
Oculinidae	1						1
Merulinidae	2		2				
Pectiniidae	3	2	1	1		1	
Siderastreidae	4	4				4	
Dendrophylliidae	5	5		1	1	3	
Coscinaraeidae	7	5		1		5	1
Fungiidae	7	3				3	4
Pocilloporidae	7	6				6	1
Agariciidae	9	8		1		7	1
Mussidae	11	9		2	1	7	1
Poritidae	16	16			7	9	
Acroporidae	24	22		2	1	19	2
Faviidae	53	49	9	2	22	16	4
	151	130	12	11	32	80	16

Table 6. Coral species Red list categories recorded in KMNR and their phylogenetic literature coverage.

RED-LIST	No. of species in kiunga	No. of species studied	Included in all (6)	Most (4-5)	Few (2-3)	Just (1)	Not included
XX	**13**	**2**			**2**		**11**
DD	**2**	**2**				**2**	
LC	**56**	**52**	7	7	**8**	**30**	**4**
NT	**48**	**48**	5	**2**	**17**	**24**	
VU	**31**	**30**		1	**5**	**24**	**1**
EN	**1**	**1**		1			
	151	**135**					

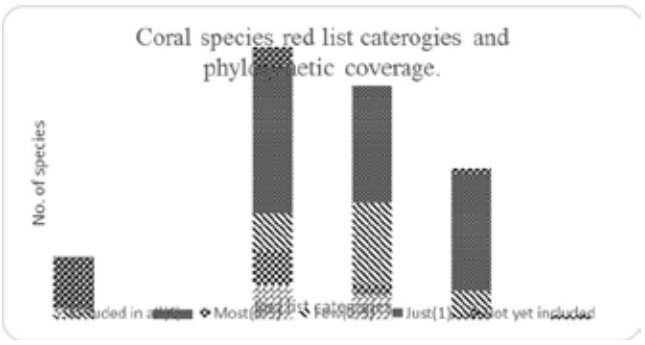

Fig. 6. Red list categories showing the number of species: included in all (6), most (4-5), few S (2-3), just one (1) or not included in the studies.

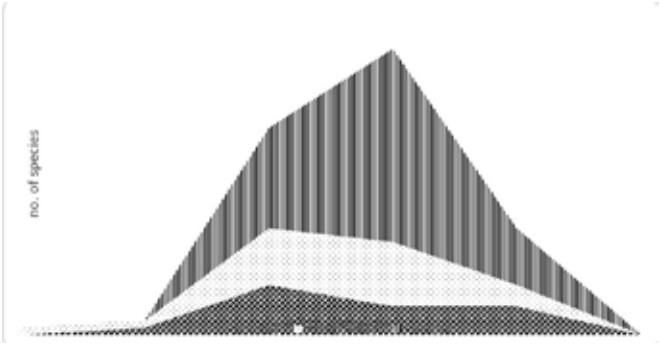

Fig. 7. Graph of top three families in KMNR, their total number of species studied and their Red list categories.

DISCUSSION

From the above results it is clear that there is a substantial body of morphological and genetic literature, table 1, that could be used as reference to report current Coral status in KMNR. This revision has been successful in confirming some priority changes in KMNR Coral species grouping; common families recorded, well studied, concentration of threatened species and gap for future projects. In this study a total of 15 families and 151 species were reviewed. The most common families are the Faviidae, Acroporidae and Poritidae.

Well to Poorly Phylogenetically Covered Coral Species

Of the top three families in KMNR Fig. 4, the Faviidae has highest number of species recorded (35%), followed by Acroporidae 16% and Poritidae 11% third. Out of these families, the best covered in the phylogenetic literature is Poritidae 100%, followed by Faviidae 92.5% and lastly Acroporidae with 91.7% while Hydrozoa and Oculinidae are poorly covered 0% .

On individual studies Fig. 3, Faviidae has the best species covered phylogenetically with 63% species being reviewed by more than half the literature publications analyzed [(6)+(4-5) + (2-3)], followed by Poritidae 44%. Poorly covered families are Hydrozoa and Oculinidae where only single species of each has been identified in KMNR and neither of the species has been reviewed based on the chosen literature in this analysis.

The Acroporidae, though with second highest number of species recorded, most of its species 86%, have been reviewed by a single publication which focused on threatened Coral species of the world (Huang, *et.al*, 2012).

Using the two Corals classifications method, morphological and genetic analysis, it has proved to be effective in refining the Coral classification status of KNMR and further allowing for analysis involving a group of factors such as common families recorded, well studied species phylogenetically, Fig. 2 and 3. Lack of phylogenetic literature data on Hydrozoa and Oculinidae could be attributed to few studies or rarity of the species in the area and this calls for rigorous studies focusing to fill this gap.

The Red List Category

Analyzing the IUCN threatened category species Fig. 2, the top three common categories recorded in KMNR are; least concern (LC) which has the highest number of species recorded (37.1%) followed by near threatened (NT) 31.8% and thirdly vulnerable (VU) 20.5%. Out of these three categories, the best covered in the phylogenetic literature Fig. 4, is near threatened 100%, followed by vulnerable 96.8% and lastly least concern with 92.9% while those not evaluated being poorly covered 15.4%.

Species in category near threatened are the best covered phylogenetically with 50% being reviewed by more than half the literature analyzed [(6) + (4-5) + (2-3)], followed by least concern and vulnerable, 42% and 20% respectively. The poorly covered are the species in data deficient categories with only one publication reviewing the two species recorded in it, Fig.5.

The total number of species recorded and not evaluated (NE) for the red list categories in KMNR is 8.6% and out of this, 84.6% is not phylogenetically covered in any of the six publication used in this study analysis. This shows a clear linkage between genetic and morphological revolutions of Coral classification where by both methods play key role in the grouping of species during current century.

The fact that there are Corals not evaluated for red listing but has been identified means there is a possibility to use the available resources to map them presently in correct groups to avoid contradictions in the future. The literature is enough to replicate the research to get enough data on these species for enhanced management.

To precisely point the pattern of distribution or risks factors in Corals, the use of genetic traits becomes important, as it places species to more critically revised groups (Huang, 2012). This analysis together with the morphological traits helps experts place Corals correctly in accordance to the susceptibility risk factors such as extinction, bleaching, diseases and distribution. The red-list for example, uses this method of analysis to affirm the extinction risk of the Corals species and which have led to the different IUCN threatened species categories; not evaluated, data deficient, least concerned, near threatened, vulnerable and endangered (IUCN, 2011).

Common Families, Phylogenetic Coverage and Red List Categories

Faviidae has the highest number of species phylogenetically studied (56%) Fig.6 and also comprises 53% of threatened species (VU+EN). Acroporidae is second with highest number of species studied 25% and including 20% of threatened species. Lastly Poritidae is the third highly studied with 18% of all species but has more threatened species 26% compared to Acroporidae.

According to IUCN, threatened species, vulnerable and endangered can be justified for localized efforts for propagation and reintroduction back to the wild to supplement the current population. Successful Coral propagation is based on using genetically and morphologically viable polyps which are able to either withstand the current threat; pollution, spawning adequately and recovers fast when damaged by the crown-of-thorns star fish (IUCN RED LIST, 2011).

Anomastrea irregularis in the family Coscinaraeidae, is listed as a vulnerable species and is rare in the KMNR. It favours turbid and muddy environment but there is an increase loss on these habitats from activities such as overfishing, pollution and outbreaks of crown-of-thorns starfish which eats Corals. *Anomestraea irregularis* can be used as an example, or candidate species for developing specific conservation measures linking

MPAs, fishery management and community conservation and development (IUCN RED LIST, 2011).

CONCLUSION

It is clear that there is a substantial body of phylogenetic literature that could be referenced for changing the traditional mode of reporting KMNR Coral classification status which mostly uses morphological traits. More than half of the recorded species in the area have been covered by phylogenetic literature which makes it possible to use this published data to further analyze critical Corals status such as extinction rates. When enough time and resources are vested in preparing descriptive taxonomic data, then regular and intensive updates addressing Coral species can be achieved. Corals are an important resource and need this regular review to enable for reclassification, protection and management planning, for example, with species observed to be data deficient. Morphological and genetic analysis should be key to understanding the changes that occur to Corals with genetic analysis forming a critical role in fine detail examination on the Coral factors such as resilience and propagation. The results shown in this study is part of a broader series of studies on Coral systematics and distributions in the WIO, which when combined will give very fine details on threat status of Coral species found in the WIO region.

REFERENCES

Arrigoni, A., Stefani, F., Pichon, M., Galli, P. and Benzoni, F. (2012). *Molecular phylogeny of the robust clade (Faviidae, Mussidae, Merulinidae, and Pectiniidae): An Indian Ocean perspective*. Molecular Phylogenetic and Evolution 65 (2012) 183–193

Budd, A., Romano, S., Smith, N., and Barbeitos, M. (2010). *Rethinking the Phylogeny of Scleractinian Corals: A Review of Morphological and Molecular Data*. Integrative and Comparative Biology, volume 50, number 3, pp. 411–427doi:10.1093/icb/icq062.

Budd, A and Stolarski, J. (2011). *Corallite Wall and Septal Microstructure in Scleractinian Reef Corals: Comparison of Molecular Clades within the Family Faviidae.* Journal of Morphology 272: pp. 66–88.

Budd, A., Fukami, H., Smith, N. and Knowlton, N. (2012).*Taxonomic classification of the reef Coral family Mussidae (Cnidaria: Anthozoa: Scleractinia).* Zoological Journal of the Linnean Society, 2012, 166, 465–529.

Cesar, H. S. (2002). *Coral reefs: their functions, threats and economic value*.

Church, J., and Obura, D. (2006). *Sustaining Coral Reef Ecosystems and their Fisheries in the Kiunga Marine National Reserve, Lamu, Kenya*. Proceedings of the 10th International Coral Reef Symposium, 1381-1390.

Faure, G., Pichon, M., Geynet, Y. (2008). *List of scleractinian Coral species cited from the Mascarene Archipelagobased on Faure (1982), updated.*

Fukami, H. Chen. C, Budd, A., Collins, A., Wallace, C., (2008). *Mitochondrial and Nuclear Genes Suggest that Stony Corals Are Monophyletic but Most Families of Stony Corals Are Not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). PLoS ONE* 3(9): e3222. oi:10.1371/journal.pone.0003222.

Fukami, H., Budd, A., Paulay, G., Sole-Cava, A., Chen, C., Iwao, K., Knowlton, N. (2004). *Conventional taxonomy obscures deep divergence between Pacific and Atlantic Corals.* Nature 2004, 427:832-835.

Fukami, H. (2008). *Short review: molecular phylogenetic analyses of reef Corals*. Galaxea, Journal of Coral Reef Studies, 10(2), 47-55.

Hamilton, H. G., & Brakel, W. H. (1984). *Structure and Coral fauna of East African reefs*. Bulletin of Marine Science, 34(2), 248-266.

Huang, D., Licuanan, W. Y., Baird, A. H., & Fukami, H. (2011). *Cleaning up the'Bigmessidae': Molecular phylogeny of scleractinian Corals from Faviidae, Merulinidae, Pectiniidae and Trachyphylliidae.* BMC Evolutionary Biology, 11(1), 37.

Huang, D. (2012). *Threatened reef Corals of the world*. PLoS One, 7(3), e34459-e34459.

Huang, D., Benzoni, F., Arrigoni, R., Baird, A. H., Berumen, M. L., Bouwmeester, J., & Budd, A. F. (2014). *Towards a phylogenetic classification of reef Corals: the Indo Pacific genera Merulina, Goniastrea and Scapophyllia (Scleractinia, Merulinidae).* Zoologica Scripta, 43(5), 531-548.

IUCN (2011). *IUCN Red List of Threatened Species.* Version 2011 2.

Kitahara, M. V., Cairns, S. D., Stolarski, J., Blair, D., & Miller, D. J. (2010). *A comprehensive phylogenetic analysis of the Scleractinia (Cnidaria, Anthozoa) based on mitochondrial CO1 sequence data.* PloS one, 5(7), e11490.

Kitano, Y. F., Benzoni, F., Arrigoni, R., Shirayama, Y., Wallace, C. C., & Fukami, H. (2014). *A phylogeny of the family Poritidae (Cnidaria, Scleractinia) based on molecular and orphological analyses.*

Kumaraguru, A. K., Jayakumar, K., & Ramakritinan, C. M. (2003). *Coral bleaching 2002 in the Palk Bay, southeast coast of India*. CURRENT SCIENCE-BANGALORE-, 85(12), 1787-1792.

KWS. (2001). *Management plan for Kiunga Marine National Reserve*.

McClanahan, T. R., Cinner, J. E., Maina, J., Graham, N. A. J., Daw, T. M., Stead, S. M., ... & Polunin, N. V. C. (2008). *Conservation action in a changing climate*. Conservation Letters, 1(2), 53-59.

Obura, D. O., Church, J., Mwadzaya, H., Wekesa, E., & Muthiga, N. A. (1998). *Rapid Assessment Of Coral Reef Biophysical And Socioeconomic Conditions in the Kiunga Marine Reserve, Kenya: Methods Development and Evaluation*. FAO/UNEP.

Obura, D., Uku, J. N., Wawiye, O. P., Mwachireya, S., & Mdodo, R. (2000). *Kenya, reef status and ecology*. Coral reef degradation in the Indian Ocean. Status Report.

Obura, D.O., Tamelander, J., & Linden, O. (Eds) (2008). *Ten years after bleaching –facing the consequences of climate change in the Indian Ocean.* CORDIO Status Report 2008.

Obura, D., Chuang, Y., Olendo, M., Amiyo, N., Church, J., et al. (2007). *Relict Siderastrea savignyana (Scleractinia: Siderastreidae) in the Kiunga Marine National Reserve, Kenya.* Zoological Studies 46: 427.

Obura, D., Amiyo, N., Mike Olendo, M. and Visram, S. (2007). *Coral Reef Monitoring update, 2004-6. Kiunga Marine National Reserve, Kenya*. CORDIO East Africa/WWF East Africa Regional Programme Office/Kenya Wildlife Service.

Church, J., & Obura, D. O. (2006). *Sustaining Coral reef ecosystems and their fisheries in the Kiunga Marine National Reserve, Lamu, Kenya*. International Coral Reef Symposium.

Obura, D. (2012). *The Diversity and Biogeography of Western Indian Ocean Reef-Building Corals.* PLoS ONE 7(9): e45013. doi:10.1371/journal.pone.0045013.

Obura, D, and Church, J. (2004). *Coral Reef Monitoring in the Kiunga Marine Reserve, Kenya. 1998-003.* CORDIO East Africa/WWF East Africa Regional Programme Office.

Secretariat of the Convention on Biological Diversity, (2010). *Biodiversity outlook 3*. Pg 46-50

Spalding, M. D., & Jarvis, G. E. (2002). *The impact of the 1998 Coral mortality on reef fish communities in the Seychelles*. Marine Pollution Bulletin, 44(4), 309-321.

UNEP (2012). *Report of The Southern Indian ocean Regional workshop to facilitate the description of ecologically or biologically significant Marine Areas.* UNEP/CBD/RW/EBSA/SIO/1/4.

Van Woesik 2003. Scleractinian taxonomy. *Corals of the Western Indian Ocean. 1-13*

Veron, J. E. N. (2000). *Corals of the World, vol. 1–3*. Australian Institute of Marine Science, Townsville.

Wallace, C. C., & Muir, P. R. (2005). *Biodiversity of the Indian Ocean from the perspective of staghorn Corals (Acropora spp).* Indian Journal of Marine Sciences, 34(1), 42-49.

Wanyonyi, I. N., Obura, D. A. V. I. D., & Malleret-King, D. E. L. P. H. I. N. E. (2008). *Coastal communities adaptation and resiliency to vulnerability: An analysis of livelihood activities in Kenya.* CORDIO Status report.

WWF 1996. *Conservation and Development of the Kiunga Marine Reserve Area Kenya*. Project KE0087.

Influence of Salt Works' Hyper-Saline Waste-Brine on Distribution of Mangrove Crabs (Decapoda) within the Gongoni-Kurawa Intertidal Area, Kenya

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Abstract

Evaporation is one of the oldest methods employed in sea salt production, a process that involves pumping seas water into a series of ponds where solar evaporation concentrates it into brine, and precipitates the salt; which is then harvested manually. Lack of baseline information on the effect of the discharged brine at the Gongoni-Kurawa region caused the need to determine its impact on the marine ecosystem. The objective of this study was to identify the effect of brine on mangrove crab species diversity and distribution. The impact of the discharged hyper-saline waste-brine waters on the mangrove crabs along the intertidal habitats bordering two of the biggest salt works; Krystalline and Kurawa salt industries was investigated in this study. Sampling was done during both spring and neap season in the year 2015 between the month of February to June using $1m^2$ quadrats, and crabs collected from the quadrats used to estimate the densities. A total of 34 mangrove crab species were recorded, with abundances significantly higher ($p>0.05$) within the inlets' habitats as compared to the outlets (discharge-point) habitats. Six species were most dominant and occurred in all of the transect samples and they were *Machrophthalmus grandidieri*, *Uca chlorophthalmus*, *Terebralia palustris*, *Machrophthalmus latreillei*, *Uca tetragonon* and *Amaea acuminata*. Higher species diversity and evenness were recorded in inlet habitats at Kurawa compared to the outlets. The inlet habitats reported higher Maximum Shannon-Wiener diversity, whereas outlets recorded lower diversity, with Marereni recording considerably lower Hmax, at 0.95. Species distribution showed a significant reduction of the genera *Uca* and *M. grandidieri* ($p<0.05$) at Marereni outlet habitats, but an increase in *U. vocans* at the inlet habitats. Similarly, there was a higher abundance of genera *Uca*, *M. ovalina*, *M. grandidieri*, *M. latreillei*, *Amaea acuminata* and *Cerithidea decollata* ($p<0.05$) during the spring tide period while the abundances of *U. vocans* dropped during the same period. This asymmetric distribution between inlets and outlets was explained by significant variations in salinity as well as site specific salinity gradients at the two study sites; Marereni and Kurawa in north coast, Kenya. Suggestions for improving salt production and quality while minimizing adverse environmental effects were recommended.

Keywords: Inter-tidal habitats, Salinity, Waste-brine, Crab

INTRODUCTION

Waste-brine discharge is one of the most significant environmental issues associated with solar sea salt production due to the presence of highly potent salt concentration and residual chemicals, (Danoun, 2007; Dawoud, 2012) including chemicals used during the 'cleaning' of the salt after harvesting. In many salt industries, and especially within the Developing and Least Developing Countries (LDCs), majority of salt industries discharge (Ochiewo, 2004) the untreated waste-brine back into the mangrove habitats along the coast with little regard to the likely impacts of these toxic concentrates on the environment and the associated flora and fauna. In Kenya, along the Gongoni-Kurawa coastal stretch in the north of Malindi, the operations of the numerous salt works are no different (Ochiewo, 2004), and the impacts of the discharges on the marine environment, although little documented, cannot be understated. The impacts can be deduced from the numerous conflicts between the salt industries and the local communities, who have highlighted the issues such as the salination of underground freshwater, mortality of both juvenile fish and adult fish species and crustaceans during weeks when the salt works discharge their brine, and when the mangroves habitats get flooded with the waste brine.

Furthermore, the mangrove areas form important nursery and feeding grounds for many offshore marine fish species as well as habitats for a variety of terrestrial birds which depend on the abundance of the marine flora and fauna in these habitats (Cohen, 2010). Consequently, any impacts on the mangrove ecosystems and coastal habitats go beyond localized species depletions (Cohen, 2010; Cooley & Heberger, 2013), to wide ecosystem impacts on the associated flora and fauna of inshore environments (Kumar & Khan, 2013), as well as both the small-scale inshore- and offshore- industrial fisheries which are closely linked to the habitats, including the bottom trawl shrimp fisheries of the adjacent Malindi-Ungwana Bay. Therefore, the little benefits derived by the locals from employment in the salt works and the scanty fisheries within the inlet reservoirs, cannot compensate for the likely wider impacts of the waste-brine discharge into the intertidal ecosystems. Evidently, the lack of baseline studies and documented information on the impacts of these salt works remains the biggest hindrance to the design of environmentally sound and sustainable salt works management systems, as well as establishment of key environmental audit guidelines for these

expansive industries. The few studies conducted within the salt works only assessed the socio-economic impacts of the salt works on the local communities (Ochiewo, 2004). Therefore, a comprehensive assessment of the salt works in relation to impacts on the mangrove ecosystems and the associated flora and fauna, as well as to the overall impacts on the environment, is long overdue. Therefore, the present study aimed to assess the impacts of the hyper saline waste-brine discharge on the environment using mangrove crabs as the key macro-invertebrates indicators. crabs are among benthic macro-invertebrates that are impacted by the physical, chemical, and biological conditions of the environments due to their limited ability to escape pollution (Ngo-Massou *et al.,* 2012), and therefore, present very good indicators of the quality of the aquatic environments (Rader & Reed, (2005). Furthermore, they exhibit stress related to the effects of both short and long term pollution events, and they may also show the cumulative impacts of pollution.

MATERIALS AND METHODS

Study Area

The study was conducted at two areas in Gongoni-Kurawa area, north coast of Kenya adjacent to the salt works as shown in the Fig. below, namely: Marereni and Kurawa. These are home to some of the biggest salt works, mangrove and benthic macro-invertebrates such as crabs of along this coast. Marereni is an area that is populated with people and whose geographical coordinates are 2° 52' 9" South, 40° 8' 44" East, whereas Kurawa is a semi-populated area with latitude and longitude coordinates of 2°41'41.28"S and 40°9'35.58"E.

Fig. 1: Map showing the sites for data collection, Marereni and Kurawa along Malindi-Ungwana Bay, in North Coast Kenya.

Data Collection

Assessments were conducted for a total of five months between February and June of the year 2015 in the two sampling stations: Marereni and Kurawa. At the two stations, crab samples were collected at both neap and spring tide. Crab sampling was done using a 1m² (1m x 1m) wooden quadrat at each site (outlet and inlet area) at the littoral zones. In order to give a representative data 10 replicate sampling locations were randomly chosen at a distance of 0-10m horizontally along the water channels that flows into the ocean and then 0-10m vertically to identify the species type, density, richness and diversity. The crab samples were hand-picked while some were scoped. The crabs were washed through a sieve, sorted and identified to species level according to Richmond, (2011) in situ and others packed in zipped paper bags and labelled depending on the site collected; and preserved in iced cooler box and taken to a laboratory for further identification according to Richmond (2011). At the laboratory the crabs were removed and counted and the number recorded based on the species type. The crab size was also determined by measuring the carapace length using a vainer calliper to the nearest 0.1centimetres. Only the record of the four most abundant crab species in both the sites was used to determine the size frequency distribution of the crabs.

Data Analysis

Data analysis was carried out based on the random sample collected during the study. Means, Standard Errors and tables were used for descriptive statistics. Data analysis was based on species level in order to provide greater resolution of selectivity and potential overlap among sites. Logistic regression was used to estimate coefficients (parameter estimates), standard error of the coefficients, z-values and p-values. These sets of parameter estimates, gave nonparallel lines for the response values. The taxa richness (for higher classification level), Shannon-Wiener diversity index -, and dominance index were used in order to reduce the multivariate (multi-taxa) complexity of the data into a single (or small number of indices) that were evaluated for each sample. Size distribution by species was established from the sampled catch using standard length measurements. Macro invertebrates species composition data was used to determine species abundance between the two sampling station; of Kurawa and Marereni.

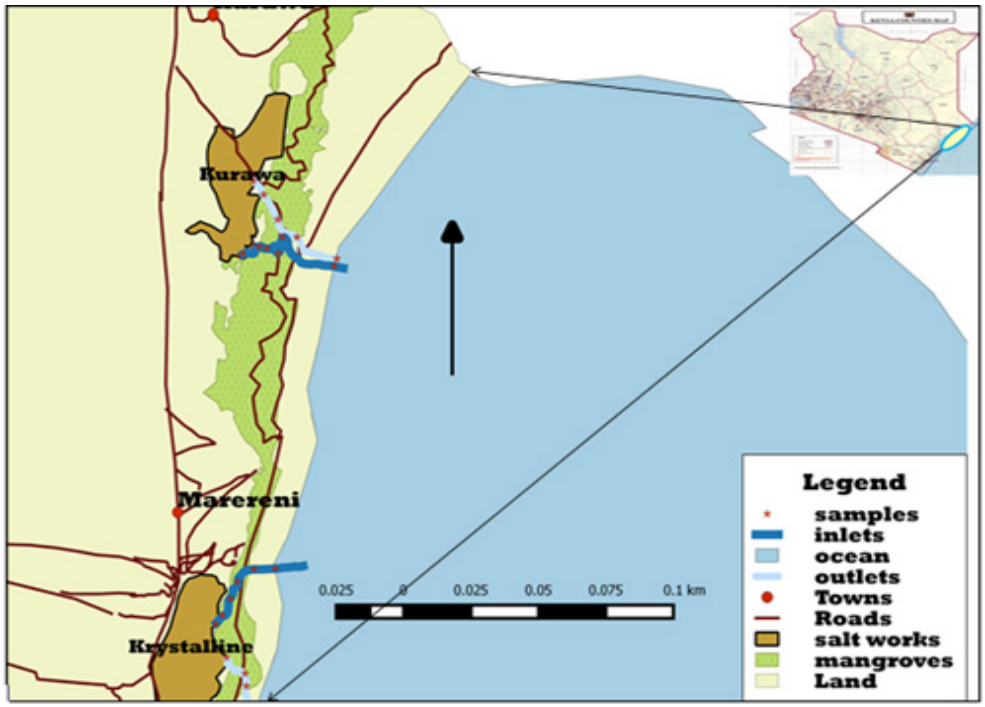

Fig. 1: Map showing the sites for data collection, Marereni and Kurawa along Malindi-Ungwana Bay, in North Coast Kenya.

RESULTS

Distribution of Crabs

A total number of 34 crab species of 17 families were captured at Marereni and Kurawa (Table 1). A total of 10 specific species were present at both the inlet and outlet both in Kurawa and Marereni. Marereni and Kurawa inlet with 24 species recorded the highest while Kurawa outlet with 19 species recorded the least. Marereni outlet recorded 21 species. Species from family Littorinidae, Macrophthalmidae, Mactridae, Ellobiidae and Grapsidae were not recorded in Kurawa outlet compared to Marereni.

Table 1: Families and macro-invertebrate species obtained at Marereni and Kuruwa area (Inlet and Outlet) with + showing presence

Family	Species	Marereni		Kurawa	
		Inlet	Outlet	Inlet	Outlet
	Alpheus sp	+		+	+
Epitoniidae	*Amaea acuminata*	+	+	+	+
Cerithiidae	*Rhinoclavissinensis*			+	+
	Cerithedea decollata		+		
	Diola lauta	+		+	
Diogenidae	*Clibanarius virescens*			+	+
	Diogenes avarus		+		
Dotillidae	*Dotilla fenestrate*	+	+	+	+
Grapsidae	*Metopograpsus messor*		+	+	+
	Llyograpsus paludicola	+	+		
Lucinidae	*Anodontia edentula*	+			
Gecarcinidae	*Cardisoma carnifex*	+			+
Potamididae	*Terebralia palustris*	+	+	+	+
	Cerithidea decollata	+		+	
Sesarmidae	*Sesarmops impressus*		+	+	+
	Chiromantes eulimene	+	+	+	+
	Neosarmatium meinerti	+	+	+	
Littorinidae	*Littoraria scabra*	+	+		
	Littoraria undulate	+	+		
	Littoraria glabrata			+	
Macrophthalmidae	*Macrophthalmus latreillei*	+		+	
	Macrophthalmus grandidieri	+	+	+	+
Mactridae	*Mactra ovalina*		+	+	
Ellobiidae	*Melampus sp*	+		+	
Varunidae	*Pseudograpsus elongate*	+	+		+
Portunidae	*Scylla serrate*	+	+	+	+
Ocypodidae	*Uca annulipes*	+	+		+
	Uca chlorophthalmus	+	+	+	+
	Ocypode ceratophthalmus			+	
	Uca inversa	+	+	+	+
	Uca tetragonon	+	+	+	+
	Uca urvillei	+	+	+	+
	Uca vocans	+	+	+	+

Species Abundance

A total of fifteen most dominant crab species was captured at the two sampling sites Fig. 1. Kurawa posted nine species compared to five at Marereni. At Kurawa *Macrophthalmus grandidieri* with 1080 number of crabs was the most dominant in both the stations while *Mactra ovalina* and *Pseudograpsus elongatus* with no representation in the outlet and inlet respectively had the least crabs (Fig. 2a). At Marereni, *M. grandidieri* and *Mactra ovalina* with 639 and no crabs was the highest and the lowest respectively (Fig. 2b).

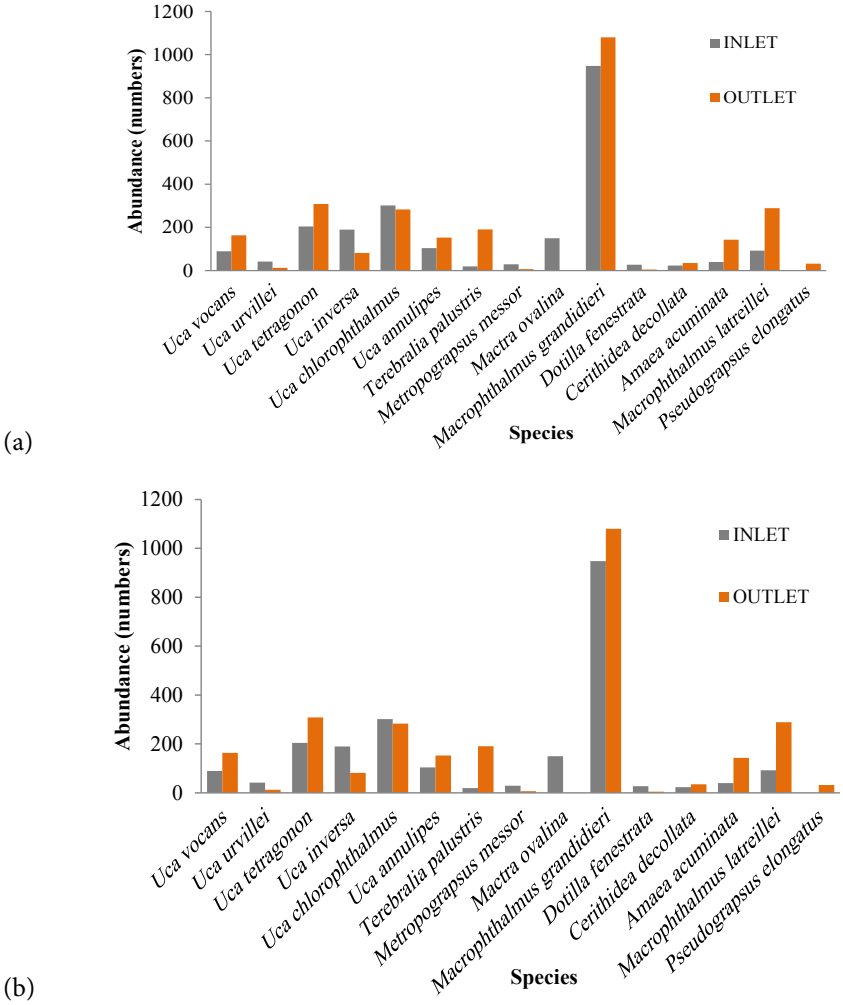

Fig. 2 : Dominant crab species at Kurawa (a) and Marereni (b) as from February to July 2015

Size-frequency Distribution

Generally *M. grandidieri*, *U. tetragonon*, *U. chlorophthalmus* and *M. latreillei* recorded the largest number in samples in every station as *U. tetragonon* recorded the least count.

In Kurawa inlet most recorded crabs were under the class interval 1-1.5 (cm); *M. grandidieri* 423, *U. chlorophthalmus* 115, *M. latreillei* 32 and *U. tetragonon* 67 number of species, while *U. tetragonon* recorded the least number under the class intervals 0-0.5 and 2.0-2.5 cm (Fig. 2).

At Kurawa outlet most of the crabs recorded were under the class interval 0.5-1.0 and 1.0-1.5 (cm). The crab *M. grandidieri*

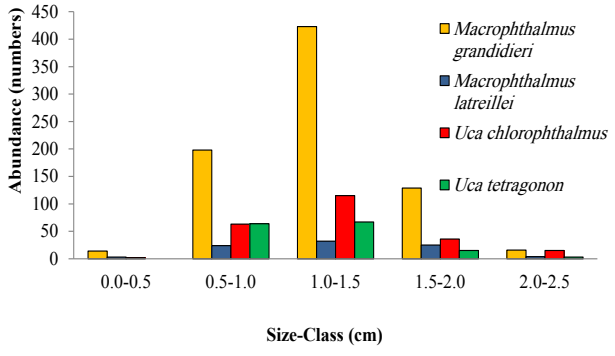

Fig. 3: The size distribution in centimetres of the four most captured crab species in Kurawa inlet.

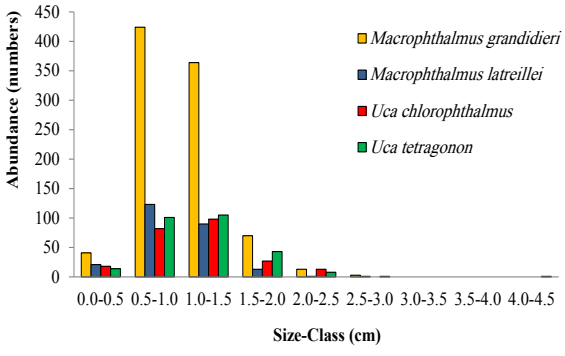

Fig. 4: The size distribution in centimetres of the four most captured crabs species in Kurawa outlet.

recorded the largest number 423 and 364 in the two class intervals respectively (Fig. 4).The lowest number of all species was recorded under the class interval 3.0-3.5 cm and above; which constituted only 0.5% in the frequency distribution.

At Marereni inlet, most crabs were caught under the class interval 0-2.5 (cm) with *M. grandidieri* crab specie having the highest record; 433. Very few numbers of specie *U. chlorophthamus* about 1% were recorded under class intervals above 0-2.5cm (Fig. 5).

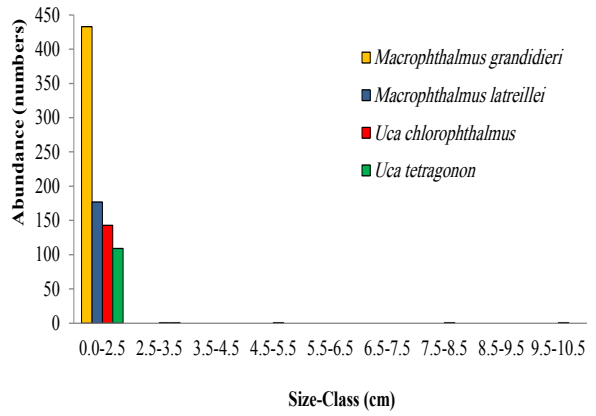

Fig. 5: The size distribution in centimetres of the four most captured macro invertebrate species in Marereni inlet.

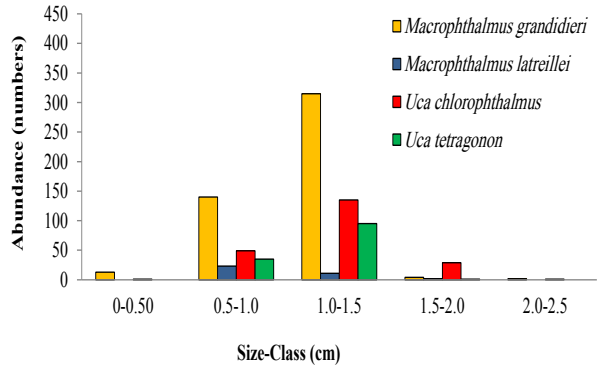

Fig. 6: The size distribution in centimetres of the four most captured macro invertebrate species in Marereni outlet.

In Marereni outlet, largest number of crabs was caught in the class interval 1.0-1.5 cm consisting of 315 *M. grandidieri*, 135 *U. chlorophthalmus* and 95 *U. tetragonon* (Fig. 8). A total of 13 crabs was caught under class interval of 0-0.5 cm while 3 crabs, two *M. grandidieri* and single *Uca chlorophthalmus* were recorded under the class interval of 2.0-2.5 cm.

Diversity Index

Table 2 : Response information used in the nominal logistic regression based on the 16 most abundant crab specie samples in the Kurawa-Marereni area of Malindi.

Diversity Index	Kurawa		Marereni	
	Inlet	Outlet	Inlet	Outlet
Shannon-Wiener	0.8912	0.8462	0.9785	1.0262
Maximum Diversity	1.1461	1.1461	1.0414	0.9542
Evenness	0.7776	0.7383	0.9396	1.0754

Logistic Regression

A total of 34 crab species were identified and enumerated in the Kurawa-Marereni area of Malindi but only 16 species with a total count of more than 200 were used in the logistic regression analysis. The most abundant species was *M. grandidieri* followed by *U. chlorophthalmus*, *T. palustris*, *U. tetragonon* and *A. acumina-ta*. The most suitable predictors of the species abundance were found to be the site (with two levels; Kurawa and Marereni), station (with two levels; inlet and outlet) and the tides (Neap and spring).

The first set of estimated logits, labelled Logit (1), are the parameter estimates of the change in logits of *U. urvillei* relative to the reference species (*U. vocans*). The p-values of <0.0005 for station, indicate that there is sufficient evidence that a change in station from inlet to outlet affected the occurrence of *U. urvillei* as compared to *U. vocans*. The negative coefficient (-3.61) for station indicates there tend to be less *U. urvillei* at the outlet as compared to the inlet. The estimated odds ratio of 0.03 implies that the odds of occurrence of *U. urvillei* at the outlet, is only 3% as compared to the inlet when site and tides are held constant (Table 3).

The p-value for site (<0.0005) indicate that there is sufficient evidence to conclude that a change in site from Kurawa to Marereni affected the occurrence of *U. urvillei* as compared to *U. vocans*. The positive coefficient (1.20) for site indicates there tend to be more *U. urvillei* at Marereni as compared to Kurawa.

The estimated odds ratio of 3.3 implies that the odds of occurrence of *U. urvillei* at Marereni is about 3 times higher than Kurawa when site and tide are held constant.

The p-value for tides (0.263) indicates that there is insufficient evidence to conclude that a change in tide cycle from neap to spring affected the occurrence of *U. urvillei* as compared to *U. vocans*. The positive coefficient (0.18288) for tides indicates there tend to be more *U. urvillei* at spring tides as compared to neap tides. The estimated odds ratio of 1.2 implies that the odd of occurrence of *U. urvillei* at spring tides is almost equal neap tides when site and station are held constant.

Table 3: Nominal logistic regression results showing the probability and odds ratio of different crab species with U. vocans as the reference even.

Predictor	Coef	SE Coef	Z	p-value	Odds Ratio	95% CI	
						Lower	Upper
Logit 1: (U. urvillei/U. vocans)							
Constant	-0.96219	0.07530	-12.78	<0.0005			
Outlet	-3.60498	0.21146	-17.05	<0.0005	0.03	0.02	0.04
Marereni	1.20202	0.13566	8.86	<0.0005	3.33	2.55	4.34
Spring	0.18288	0.16333	1.12	0.263	1.20	0.87	1.65
Logit 2: (U. tetragonon/U. vocans)							
Constant	0.41962	0.04533	9.26	<0.0005			
Outlet	-0.26164	0.05027	-5.20	<0.0005	0.77	0.70	0.85
Marereni	1.59419	0.07726	20.63	<0.0005	4.92	4.23	5.73
Spring	1.62580	0.06452	25.20	<0.0005	5.08	4.48	5.77
Logit 3: (U. inversa/U. vocans)							
Constant	-0.00679	0.05124	-0.13	0.895			
Outlet	-2.13681	0.06255	-34.16	<0.0005	0.12	0.10	0.13
Marereni	1.30951	0.08532	15.35	<0.0005	3.70	3.13	4.38
Spring	2.47296	0.07161	34.53	<0.0005	11.86	10.30	13.64
Logit 4: (U. chlorophthalmus/U. vocans)							
Constant	0.54079	0.04381	12.34	<0.0005			
Outlet	-0.31533	0.04833	-6.53	<0.0005	0.73	0.66	0.80
Marereni	1.66802	0.07584	21.99	<0.0005	5.30	4.57	6.15
Spring	2.18751	0.06294	34.76	<0.0005	8.91	7.88	10.08
Logit 5: (U. annulipes/U. vocans)							
Constant	0.40732	0.04919	8.28	<0.0005			
Outlet	-0.78605	0.05810	-13.53	<0.0005	0.46	0.41	0.51
Marereni	0.85807	0.08903	9.64	<0.0005	2.36	1.98	2.81
Spring	-0.62971	0.09882	-6.37	<0.0005	0.53	0.44	0.65
Logit 6: (T. palustris/U. vocans)							
Constant	0.60076	0.04608	13.04	<0.0005			
Outlet	0.63521	0.05091	12.48	<0.0005	1.89	1.71	2.09
Marereni	1.26588	0.07690	16.46	<0.0005	3.55	3.05	4.12
Spring	-0.59512	0.07213	-8.25	<0.0005	0.55	0.48	0.64
Logit 7: (S. impressus/U. vocans)							
Constant	-7.99744	0.35593	-22.47	<0.0005			
Outlet	3.71150	0.29465	12.60	<0.0005	40.92	22.97	72.89
Marereni	6.17567	0.21990	28.08	<0.0005	480.91	312.52	740.01
Spring	-2.53461	0.29751	-8.52	<0.0005	0.08	0.04	0.14
Logit 8: (P. elongatus/U. vocans)							
Constant	-2.17558	0.12415	-17.52	<0.0005			
Outlet	2.14976	0.12776	16.83	<0.0005	8.58	6.68	11.02
Marereni	-0.43898	0.14061	-3.12	0.002	0.64	0.49	0.85
Spring	-9998.80	4746.06	-2.11	0.035	0.00	0.00	0.00

All the species showed evidence of differences from *U. vocans* between site, station and tide cycle except *D. fenestrata* between tide cycles. The odds ratio against *U. vocans* was highest for *S. impressus* between sites (480), *M. ovalina* between tide cycles (172) and *D. fenestrata* between stations (44).

Table 3: (Continued.)Nominal logistic regression results showing the probability and odds ratio of different crab species with U. vocans as the reference even.

Predictor	Coef	SE Coef	Z	p-value	Odds Ratio	95% CI	
						Lower	Upper
Logit 9: (M. messor/U. vocans)							
Constant	-1.25172	0.08442	-14.83	<0.0005			
Outlet	-2.24273	0.15176	-14.78	<0.0005	0.11	0.08	0.14
Marereni	0.43433	0.18813	2.31	0.021	1.54	1.07	2.23
Spring	-0.61441	0.25115	-2.45	0.014	0.54	0.33	0.88
Logit 10: (M. ovalina/U. vocans)							
Constant	-1.19818	0.08268	-14.49	<0.0005			
Outlet	-4.97077	0.12078	-41.16	<0.0005	0.01	0.01	0.01
Marereni	-1.45208	0.13482	-10.77	<0.0005	0.23	0.18	0.30
Spring	5.14769	0.09633	53.44	<0.0005	172.03	142.44	207.78
Logit 11: (M. latreillei/U. vocans)							
Constant	0.79768	0.04384	18.20	<0.0005			
Outlet	-0.64202	0.04943	-12.99	<0.0005	0.53	0.48	0.58
Marereni	1.84998	0.07665	24.14	<0.0005	6.36	5.47	7.39
Spring	1.06248	0.06534	16.26	<0.0005	2.89	2.55	3.29
Logit12:(M. grandidieri/U. vocans)							
Constant	2.64972	0.03905	67.85	<0.0005			
Outlet	-0.60249	0.04413	-13.65	<0.0005	0.55	0.50	0.60
Marereni	1.32654	0.07333	18.09	<0.0005	3.77	3.26	4.35
Spring	1.78184	0.06025	29.57	<0.0005	5.94	5.28	6.69
Logit 13: (D. fenestrata/U. vocans)							
Constant	-3.71402	0.12394	-29.97	<0.0005			
Outlet	1.60102	0.11487	13.94	<0.0005	4.96	3.96	6.21
Marereni	3.77995	0.10328	36.60	<0.0005	43.81	35.78	53.64
Spring	-0.02540	0.10810	-0.23	0.814	0.97	0.79	1.20
Logit 14: (C. decollata/U. vocans)							
Constant	-0.55714	0.05011	-11.12	<0.0005			
Outlet	-0.74764	0.05259	-14.22	<0.0005	0.47	0.43	0.52
Marereni	3.05420	0.07859	38.86	<0.0005	21.20	18.18	24.74
Spring	2.18144	0.06663	32.74	<0.0005	8.86	7.77	10.09
Logit 15: (A. acuminata/U. vocans)							
Constant	-0.95301	0.05728	-16.64	<0.0005			
Outlet	1.39259	0.06063	22.97	<0.0005	4.03	3.57	4.53
Marereni	2.15333	0.07692	27.99	<0.0005	8.61	7.41	10.02
Spring	1.07519	0.06568	16.37	<0.0005	2.93	2.58	3.33

The Log-Likelihood from the maximum likelihood iterations with G-statistic (the test statistic for testing the null hypothesis that all the coefficients associated with predictors equal 0 versus them not all being zero) was 48.4 with a p-value of <0.0005, indicating that at

a = 0.05, there is sufficient evidence for at least one coefficient being different from 0 (Table 4).

Pearson and deviance goodness-of-fit tests gave p-values 0.730 and 0.640 respectively, indicating that there is evidence to suggest the model fits the data. If the p-value is less than selected a-level, the test would indicate that the model does not fit the data.

Table 4: *The Log-Likelihood and Goodness of Fit test for the nominal logistic regression model of the crab species.*

Log-Likelihood = -201176.098			
Test that all slopes are zero: G = 48399.994			
DF = 45			
p-value = <0.0005			
Goodness of Fit Test:			
Method	**Chi-Square**	**DF**	**p-value**
Pearson	5.97083E+14	270	0.730
Deviance	1.84772E+05	270	0.640

DISCUSSION

Distribution and Diversity

This study provided a starting point for the distribution, abundance and diversity of benthic fauna of mangroves in Kurawa-Marereni area. The distribution of crabs showed relationships to salinity and degree of tidal in unration. The dominant species shifted throughout the study period. These observed differences among the crabs may have been related to changes in water quality which affect the environment causing change. The effect of the brine waste discharge in water quality of the water channels in Kurawa and Marereni were also evident in the crab communities, which exhibited reduced species numbers and individuals per species per site based on the logistic regression test done. Such species can therefore be used as indicators of salt intrusion since they can tolerate the hyper-saline water. This is in line with Danoun (2007), who reported that changes in the salinity can play two opposite roles on the marine organisms' existence; it can be of benefit for some of these organisms such as shellfish and at the same time can have an adverse impact on other species. Decrease in the abundance of majority of crabs in this study associated with tides at different stations showed indeed how the brine discharge seasonal alters the abundance of the crabs, since salinity levels change with tidal flows. This was similar to what Savenije of 2006 found.

The change of the salinity levels could probably be caused by brine waste discharge, which has been found to be associated with reduction in the density of less tolerant species (Von Medeazza, 2005), and increase in the density of more tolerant species. Literature (Cohen, 2010; Hossain *et al.,* 2015), also documents that the coastal areas, salinity can be viewed as the major factor limiting species distributions as these vary significantly in relation to the level of salinity.

Water salinity changes also have an influence in the population density of the crabs, causing higher population growth rate. Marine animals inclusive of the crabs are adapted to keep their body salts at a constant level, so that they don't interfere with the metabolism within cells, but significant changes in salinity can cause problems for some and also can negatively affect their growth and reproduction, and ultimately, their survival; which was same with what was found in this study (Orr *et., al* 2005; Doney *et., al* 2012), hence recording different numbers of species at the outlets compared to the inlets. This explains how some of the crabs in this study were able to cope with large salinity fluctuations while others tolerated a narrow range of salinities, hence conquering with the fact that brine constitutes a hyper-saline layer that sinks towards the seabed and due to its greater density imposes great impact on the benthos (Von Medeazza, 2005).

Diversity values in the study area ranged from 0.7383-1.1461. These values suggested that the mangroves ecosystem under which the crabs were examined in this study were heavily polluted and the crab community was under stress due to either natural or anthropogenic factors. This explains perhaps the effect of the present brine discharged from the salt industries along the water channels that affects the crabs' diversity. Lower value of the diversity index is generally interpreted as characteristic of a less assorted population in terms of species as compared to a high value which describes high variety of species in a population. The low value also described a polluted condition in this case, a poor water quality state over time, where depending on the species few (more) tolerant genera dominate the community. Higher values were recorded in normal sea waters (inlets) where few or more specie dominate depending of the tolerant ability.

Maximum diversity recorded in this study at the inlets might be due to more favourable environmental factors, such as low salinity which play a vital role in faunal distribution (El wahab & Hamada, 2012; Kumar & Khan, 2013). The Shannon Wiener's diversity index for the crab species captured in this study was higher in Marereni outlet 1.026 than Marereni inlet 0.979, a case that was different in Kurawa. The diversity value in Kurawa was high in the inlet 0.891 as compared to the outlet 0.846. There was no major difference in terms of species diversity between the two stations (Kurawa outlet/Kurawa inlet). This explains that there are more varied species in Marereni outlet compared to the inlet which could be probably due to the presence of waste brine discharged from the salt industry, thus bringing about the existence of species that can only tolerate high levels of salinity compared to those at the inlet station. A different scenario was observed in Kurawa. Effects of brine on estuarine system have high levels of dissolved solids which allow the formation of a density gradient, especially in low energy systems such as bayous; oil and chlorides are incorporated into sediments near brine discharges (Cooley & Heberger, 2013). This condition severely depressed the abundance and richness of benthic in-fauna

(Ruso *et al.,* 2007). In addition Bryant (1990) documented that the ranges of crab species diversity were greater in Oklahoma streams than in salt Creeks.

Crab Size Distribution

Based on the four most recorded mangrove crabs species, the present study shows that there was significant difference in size in the capture of *M. grandidieri*, *M. latreillei*, *U. chlorophthalmus* and *U. tetragonon*. There was no major difference in size of *M. latreillei* captured. Most small sized crabs of large numbers were recorded at the outlets whereas small sizes of few numbers were recorded at the inlets in all sites. This could be probably due to the presence of the hyper-saline water which could be affecting the growth size of the crabs, thus causing existence of more small sized crabs at the polluted sites and less number of small sized crabs at the unpolluted sites. This is well explained by Conde *et al.,* (2000); although crabs recognitions or adjustments to polluted areas seemed to be high, some differences in size have been seen among populations at different water salinities levels. Studies have also shown that crabs found in hyper-saline lagoons appear to mature at a smaller size than those in fresher riverine areas (Zimmerman *et al*., 2002).

CONCLUSION AND RECOMMENDATION

Based on the objectives, results from this study and available literature, it is concluded that the hyper-saline waste-brine discharge affects the mangrove crab species in several ways: Crab diversity was higher in the inlet (less polluted) as compared to the outlet (more polluted) site. Few numbers of small-sized crab species; *M. grandidieri*, *U. chlorophthalmus*, *U. tetragonon* and M. latreillei, was found at the outlets as those observed at the inlets indicting effect of brine on their growth. Brine discharge seasonally alters the abundance of crabs. There was increase in abundance of crab species; *U. urvillei*, *U. tetragonon*, *U. chlorophthalmus*, *U. inversa*, *M. grandidieri*, *M. latreillei*, *M. ovaina*, *A. acuminata* and *C. decollata* compared to *U. vocans* during spring tide as compared to neap due to their physiological adaptation to high salinity levels. It can therefore be concluded that variation in species diversity, dominance and richness were as a result of spatio-temporal change of water quality. Therefore monitoring of the status and conditions of the crabs in this region was recommended, using the [Before-After Control-Impact] Monitoring]. This information will be helpful to advice the salt works managers of what levels of the brine discharge is affecting the aquatic resources specially the crabs and to what extent.

REFERENCES

Bryant, R. (1990). Species diversity of benthic macro-invertebrates in Salt Creek, Oklahoma. In *Proceedings of the Oklahoma Academy of Science* (Vol. 70, pp. 9–12). Retrieved from http://ojs.library.okstate.edu/osu/index.php/OAS/article/viewFile/5456/5082

Cohen, N. (2010). The effect of increased salinity on diversity and abundance of diatoms. *Unpublished Report. Pennsylvania State University, University Park, Pennsylvania, USA.[Online] URL: Http://Www. Marcellus. Psu. Edu/Resources/PDFs/Salinity. Pdf*. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.434.978&rep=rep1&type=pdf

Conde, J. E., Tognella, M. M. P., Paes, E. T., Soares, M. L. G., Louro, I. A., & Schaeffer Novelli, Y. (2000). Population and life history features of the crab Aratus pisonii (Decapoda: Grapsidae) in a subtropical estuary. *Interciencia*, *25*(3).

Cooley, H., & Heberger, M. (2013). Key issues for seawater desalination in California: Energy and greenhouse gas emissions. *Pacific Institute, Oakland*.

Danoun, R. (2007). *Desalination Plants: Potential impacts of brine discharge on marine life*. Retrieved from https://ses.library.usyd.edu.au/handle/2123/1897

Dawoud, M. A. (2012). Environmental impacts of seawater desalination: Arabian Gulf case study. *International Journal of Environment and Sustainability*, *1*(3).

Doney, S. C., Ruckelshaus, M., Duffy, J. E., Barry, J. P., Chan, F., English, C. A., … Knowlton, N. (2011). Climate change impacts on marine ecosystems. Retrieved from https://www.annualreviews.org/doi/pdf/10.1146/annurev-marine-041911-111611

El Wahab, M. A., & Hamoda, A. Z. (2012). Effect of desalination plants on the marine environment along the red SEA, Egypt (case study). *Int. J. Mar. Sci*, *28*(2), 27–36.

Hossain, G. M., Rahman, M. S., & Khan, S. A. (2015). Species composition, richness, density and distribution of climbers in relation to salinity in Sundarbans mangrove forest of Bangladesh. *International Journal of Chemical, Environmental & Biological Sciences (IJCEBS)*, *3*(1), 78–83.

Kumar, P. S., & Khan, A. B. (2013). The distribution and diversity of benthic macroinvertebrate fauna in Pondicherry mangroves, India. *Aquatic Biosystems*, *9*(1), 15.

Ngo-Massou, V. M., Essomè-Koum, G. L., Ngollo-Dina, E., & Din, N. (2012). Composition of macrobenthos in the Wouri River estuary mangrove, Douala, Cameroon. *African Journal of Marine Science*, *34*(3), 349–360.

Ochiewo, J. (2004). Changing fisheries practices and their socioeconomic implications in South Coast Kenya. *Ocean & Coastal Management*, *47*(7–8), 389–408.

Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., … Joos, F. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, *437*(7059), 681.

Rader, R., & Reed, S. (2005). NOTES AND NEWS. *Crustaceana*, *78*(3), 361–365.

Richmond, M. D. (2002). A field guide to the seashores of Eastern Africa and the Western Indian Ocean Islands. *AGRIS and Swedish International Development Cooperation*

Agency, Stockholm (Sweden) Eng Dar Es Salaam Univ. (Tanzania) Eng.

Ruso, Y. D. P., De la Ossa Carretero, J. A., Casalduero, F. G., & Lizaso, J. S. (2007). Spatial and temporal changes in infaunal communities inhabiting soft-bottoms affected by brine discharge. *Marine Environmental Research*, *64*(4), 492–503.

Savenije, H. H. G. (2006). *Salinity and Tides in Alluvial Estuaries* (1 edition). Amsterdam: Elsevier Science.

Sedivy, V. M. (2009). Processing of salt for chemical and human consumption. In *9th International Symposium on Salt*. Retrieved from https://www.researchgate.net/profile/Vladimir_Sedivy/publication/228505059_Processing_of_salt_for_chemical_and_human_consumption/ks/0c96052120e274db42000000/Processing-of-salt-for-chemical-and-human-consumption.pdf

Von Medeazza, G. M. (2005). “Direct” and socially-induced environmental impacts of desalination. *Desalination*, *185*(1–3), 57–70.

Zimmerman, R. J., Minello, T. J., & Rozas, L. P. (2002). Salt marsh linkages to productivity of penaeid shrimps and blue crabs in the northern Gulf of Mexico. In *Concepts and controversies in tidal marsh ecology* (pp. 293–314). Springer.

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