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RESEARCH PAPER

Integrated multitrophic aquaculture of sandfish (*Holothuria scabra*) with marine water acclimatized Nile tilapia (*Oreochromis niloticus*) and oyster (*Sacostrea cucullata*) for increased production in Kenya

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Abstract

This study compared the growth performance, production, and quality of culture environment of integrated multitrophic aquaculture (IMTA) systems with different species combinations in earthen ponds. The experiment was designed to assess the viability of using marine water acclimatized Nile tilapia (*Oreochromis niloticus*) as the fed component of the IMTA system in combination with sea cucumbers (*Holothuria scabra)* and Oysters *(Sacostrea cucullata*) as extractive species. The control (C) experiment and a monoculture of *O. niloticus*. Treatment 1 (T1) had a combination of *O. niloticus* and *H. scabra*. Treatment 2 (T2) had a combination of *O. niloticus*, *H. scabra* and *S. cucullata* and Treatment 3 (T3) had a combination of *O. niloticus* and *S. cucullata*. Stocking was 2 ind./m^2, 1.9 ind./m^2 and 2.1 ind./m^2 for tilapia, sea cucumber and oysters respectively and replicated in all the treatments. During the 150 days culture period, *S. cucullata* attained weight gain of 26.55 ± 0.26 g and 23.74 ± 2.6 g in T2 and T3 respectively. Weight gain of *H. scabra* in T1 and T2 was 146.7 ± 6 g and 153.39 ± 2.04 g respectively. The final average body weight (ABW) of the fed species *O. niloticus* was significantly higher ($p < 0.05$) in T2 at 218.82 ± 1.55 which had all the three species combinations. The findings of the study show that the IMTA that had a combination of all three species outperformed those that had two species.

Introduction

Global fish consumption has been on the increase at an annual rate of 3.1 % which is nearly twice that of yearly world population growth of 1.6 % and more than all other animal protein food like meat and milk which increase at 2.1 % per year (FAO, 2020). This has been as a result of world aquaculture production which has constantly been on the increase reaching 46 % in the year 2018 up from 25.7 % in the year 2000 (FAO, 2020). However, the rapid expansion in aquaculture has raised several concerns mainly on environmental and biosecurity issues resulting from accumulation of nutrients and pathogens in aquatic ecosystems as a result of external inputs in form of fertilizers, feeds and effluents directed to open culture systems (Skriptsova and Miroshnikova, 2011; Dong et al., 2018). A possible remedy is in situ removal of organic and inorganic nutrients which could be attained through biological means by introduction of filter feeders and deposit feeders (Neori et al., 2004, Samocha et al., 2015, Irisarri et al., 2015). This approach comprises the concept of integrated multi trophic aquaculture (IMTA) (Neori et al., 2004). In IMTA systems the waste of the main cultured species (the fed component) is utilized by the introduced extractive species which depend on it for energy and growth while at the same time cleaning the culture environment (Chopin, 2013). In addition to supporting sustainable and resilient aquaculture, integration has resulted in benefits like higher productivity, improved resource–use efficiency, and reduced environmental impacts (FAO, 2020).

In Kenya, marine aquaculture has mainly been practiced as a single species (monoculture) venture in most of the farms with most farmers growing either shrimps (*Penaeus monondon* or *Penaeus indicus)*, milkfish (*Chanos chanos*), mud crab (*Sylla seratta*) or recently Nile tilapia (*Oreochromis niloticus*) in marine environment (Mirera et al., 2022). There have also been trials of polyculture of shrimp and milkfish in intertidal ponds (Roonback et al., 2002; Mirera, 2011). The main difference between polyculture and IMTA is that whereas in polyculture the cultured organisms are both fed species sharing the same chemical and biological processes, in IMTA system the cultured organisms usually feed at different trophic levels provided by the system. The IMTA system thus, in addition, has gained of higher nutrient utilization efficiency and environmental mitigation as compared to polyculture system (Biswas et al., 2020).

Practice of IMTA has advantages of boosting production and achieving environmental sustainability, moreover the introduction of other species enhances diversification of culture species which is key towards resilience of marine aquaculture sector. Previous studies by Largo et al. (2016); Cunha et al. (2019) and Chang et al. (2020) have demonstrated viability of IMTA systems established within the intertidal zone and as well as those within sheltered bays. The studies used different species combinations of fed and extractive species. A successful IMTA system therefore requires identification of suitable combinations of species which will yield better returns on investment and mitigate some negative environmental impacts of aquaculture.

The benefits derived from integrated multitrophic aquaculture systems are both ecological and economic in nature (Chopin, 2021) and involves recovery and reuse of wastes through trophic interactions by growing valuable complementary species (Barrington et al., 2009). The farmer must therefore understand the ecological dynamics of the culture system and the organisms under culture for better performance of the enterprise. In Kenya, there has not been any studies on semi-intensive mariculture in earthen ponds where IMTA technologies have been applied. However, promising results were reported in the first IMTA trial in Kenya where shrimps (*P. indicus*) was the fed organisms with sea cucumber and cockles (*Anadara antiquate)* as extractive components (Magondu et al. (2021). The present study was designed to compare the performance of different species combinations; a herbivorous fish, a filter feeder, and a deposit feeder in earthen ponds in an IMTA setup. The overall objective was to evaluate the growth performance of three species: Nile tilapia (*Oreochromis niloticus)*, Sea cucumber (*Holothuria scabra* and oysters (*Sacostrea cucullata)*; in different species combination in an IMTA experimental setup.

Materials and Methods

Experimental design and study organisms

The study was conducted over a period of 150 days using eight rectangular earthen ponds each measuring 12 x 10 m and 1m depth hired from Umoja self-help group located in Kilifi County, Kenya (Figure 1). The study employed an IMTA design with four treatments designated as C (control), T1, T2, and T3 with different species combinations. The first treatment (i) C (control) had a monoculture of Nile tilapia, (ii) T1 had a combination of Nile tilapia and Sea cucumbers (iii) T2 had a combination of Nile tilapia, Sea cucumbers, and oysters and (iv) T3 had a combination of Nile tilapia and oysters. Each treatment had two randomly assigned replicate ponds.

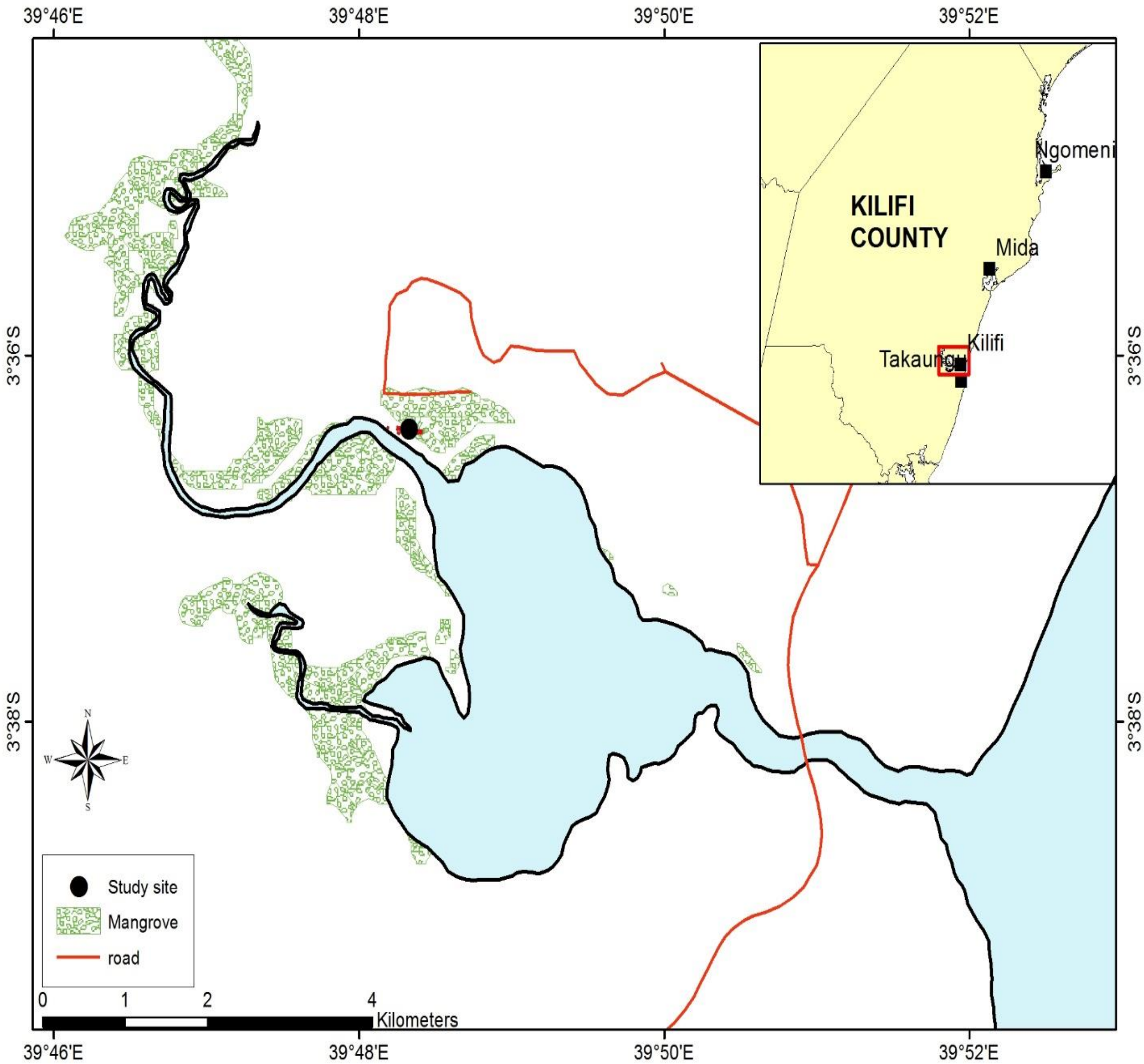

Figure 1. Site location for Umoja self-help group mariculture ponds in Kibokoni, Kilifi County, Kenya.

Preparation of experimental ponds

The experimental ponds were drained completely and sundried for a period of two weeks to eradicate predators and other living organisms. Sand was added to ponds in treatment T1 and T2 so as to make their bottom substrate allow easy burrowing by sea cucumbers as in their natural habitat environment. Lime ($CaCO_3$) was then applied to each pond bottom at 300 g/m^2 and the ponds allowed dry for 7 days. The ponds were then filled with water during high tide coming in through dug in channels from the creek. Screens were put at the inlet to prevent predators getting into the ponds. Three days after filling ponds with water, the ponds were fertilized using a combination of Urea and Diammonium Phosphate fertilizers at 3 g/m^2 and 2 g/m^2 respectively. The ponds were left for 7 days to allow growth of natural food organisms.

Collection and transportation of culture organisms

Sea cucumbers and oysters used in this study were collected from the wild while Nile tilapia were obtained from the hatchery facility at KMFRI, Mombasa. A total of 1000 juvenile sea cucumbers were collected from Vanga (4°39´S, 39°13´E) South Coast, Kenya. Collection was done by hired fishermen who hand-picked the organisms during low tide and placed them into inert polythene bags for transportation to a holding tank at KMFRI, Mombasa. A total of 1050 oyster spats settled on mangrove plant trunks were gathered by hired experienced oyster collectors during low tide from Kilifi creek (3°36´S, 39°50E) North Coast, Kenya. Collected oyster spats were placed in open basins filled with sea water for transportation to the culture baskets mounted in the experimental ponds. A total of 1950 juvenile Nile tilapia obtained from the hatchery at KMFRI Mombasa together with the acclimatized sea cucumbers were transported in separate containers to the experimental site for stocking into the culture ponds.

Stocking and management of ponds

Each pond was stocked with the culture organisms according to the stocking numbers as illustrated in Table 1 and according to the species combinations in the four treatments. Among the stocked species, Nile tilapia (*O. niloticus*) was the fed species, whereas oyster (*S. cucullata*) and sea cucumber *(H. scabra)* were the extractive species. Oysters were put in perforated plastic baskets used for shell fish culture and suspended in water column from horizontally fixed poles in respective experimental ponds following Higgins et al. (2011).

A low cost formulated feed of 30% crude protein was given as supplementary feed to the Nile tilapia which were the fed species in the IMTA setup for this study. Fish were fed at 5% body weight of the formulated feed twice daily at 9:00 AM and 3:00 PM by casting the feed over the pond surface to achieve even distribution. The quantity of feed was adjusted at 20 days interval based on the body weight of fish calculated from periodic sampling. An assumption of 90 % survival was made during the culture period, which might have occurred due to unavoidable mortality during sampling and undetected predation. Net screens were placed at the pond inlets and outlets and strings on top across the ponds to prevent bird predation. Desirable alkalinity levels, water quality, and primary productivity were maintained by monthly application of lime and inorganic fertilizer at 300 g/m^2 and 2 g/m^2 respectively. Pond water level was maintained at 1 m depth during high spring tide after compensating for evaporation and seepage.

Table 1. Number of individuals of the cultured species stocked in control (C) and IMTA treatments (T1, T2 and T3)

Species	C	T1	T2	T3
Oreochromis niloticus	240	240	240	240
Holothuria scabra		228	228	
Sacostrea cucullata			252	252

Sampling and measurement of cultured organisms

Determination of length and weight of cultured organisms was done during sampling which took place after every 20 days subsequent to the next high tide. A 20 % sample size of the stocking numbers of the different organisms was measured. The weight of 50 individuals of Nile tilapia from each replicate were measured using a digital scale (Aslor model) to the nearest 0.01g. Total length was measured to the nearest 0.01 cm on a ruler scale. A sample of 45 sea cucumbers from each replicate were handpicked, placed in small buckets to reduce shock and allow water come out of their body, where necessary paper towels were used to dry out excess water. The organisms were further measured for body length (to the nearest 0.01 cm) using a meter ruler and weighed (to the nearest 0.01 g) using a digital balance (Watanabe et al., 2014). Sampling of oysters involved hand picking of 50 pieces from each replicate pond and placing them in a basin with clean water. Shell length of the oyster was determined to the nearest 0.1 mm using a vernier caliper and later converted to cm during calculations. Individual weight of the oysters was determined on a digital weighing balance to the nearest 0.01 g (Comeau, 2013).

Determination of growth parameters such feed conversion ratio (FCR) and specific growth rate (SGR) were calculated from the data obtained. FCR is the ratio of the quantity of food distributed (g) to the weight gain of fish (g), over the culture period. This was used to judge the efficiency of feed utilization by fish for both diets. It was calculated by dividing the total amount of feed used (dry matter basis) by the weight gain. Specific growth rate (%body weight/day) was calculated using the formula, SGR = ($lnWT_F$-$lnWT_I$)*100/T where WT_F= average final fish weight (g), WT_I = average initial fish weight (g), T= duration of the experiment (days).

Harvesting of cultured organisms

At the end of the experiment all organisms were harvested; measured for total length and weighed. Each experimental pond was first drained to 0.25 m level by opening the outlets to allow the water out while retaining the screens to prevent the organisms from escaping. Nile tilapia in the control were first partially harvested using a drag net. A scoop net was then used to harvest the remaining fish. In the IMTA ponds, harvesting of the oysters was first conducted by emptying the oyster culture baskets and placing them in labeled basins with water, Sea cucumber were then harvested by handpicking from the pond bottom and placing them in separate labeled basins for later measurement and weighing. All harvested organisms were counted and recorded. Harvested organisms were sold at prevailing market rates for respective species and data recorded for economic analysis.

Determination of water quality parameters

Sampling for water quality parameters was done on monthly basis preceding the next high or low tide. The main parameters monitored were; salinity, water temperature, transparency, pH, dissolved oxygen, total dissolved solids which were done in-situ using a multi-parameter kit Hanna instruments model. Light penetration as a measure of water transparency from each sampling station was determined using a white Secchi disk. Water samples from each pond are collected for determination of Nitrite-nitrogen (NO_2-N), Nitrate-nitrogen (NO_3-N), Nitrogen-ammonia (NH_3-N) (TAN) and phosphate-phosphorous (PO_4-P) using a horizontal water sampler from three sampling points in each pond and preserving samples at 10:00hrs at each sampling day. In the laboratory, the water samples were filtered through microfiber glass filter paper (Whatman GF/C) using a vacuum pressure air pump (Shemer et al., 2017). The samples were then subjected to an auto analyser for nutrient analysis using the Continuous Flow Analysis technique following standard procedure outlined in APHA (2005).

Evaluation of economic performance

Data on expenditure and income from sale of harvested organisms were used in an economic analysis based on the developed IMTA system in comparison to the marine tilapia monoculture system. The economic performance of the treatments and their comparison were analyzed with estimation of gross returns, net income, and Cost Benefit Ratio (CBR) as shown below and according to (Biswas et al., 2012).

Net income = Total income – total expenditure

CBR was determined as CBR=Total income/total expenditure

Expenditure comprised of costs of inputs including the cost the different seeds (*O. niloticus*, *S. cucullata*, and *H. scabra*), feeds administered, labour, fertilizers, lime, netting material, and other operational costs like purchase of tools for pond maintenance, payment for security hire and pond attendant. The cost of production was estimated based on local market value price in the current US Dollar equivalence (1USD =105 Kenya currency). Produced crop of marine tilapia was sold at the local market outlet and in hotel, oysters were harvested for local consumptions at farm level while taking into consideration their market price while the sea cucumbers were sold to a local dealer for export. Total return from the crop produced was estimated by price of organisms sold. Gross margin was estimated by subtracting the total production cost from the total return. The costs did not include the initial investments but rather the routine farm operations. The outcome of the economic analysis was used to determine the viability of both systems.

Statistical analyses

Comparisons of the different IMTA treatments and the control were analyzed by calculating the mean and standard error of the mean of length and weights of replicated biomass using the descriptive statistics tool in Microsoft Excel 2013. Comparisons for water quality parameters among the four treatments was done by one-way ANOVA and repeated measures ANOVA respectively followed by post hoc Tukey HSD test (Zar, 1998). Data were checked for normality and homogeneity of variance using Shapiro-wilk's and Levene's tests respectively. Statistical analyses were done using Excel version 2013 and SPSS statistical software IBM version 22. In all statistical testing, differences were considered to be statistically significant at $p < 0.05$.

Results

Growth performance of the cultured organisms among the different treatments

The final average body weight (ABW) of the fed species (Nile tilapia) was significantly higher ($p < 0.05$) in T2 which had a full integration of both fed and extractive species. The other two IMTA treatments (TI and T3) showed higher final ABW of the fed species as compared to the control which had a mean of 160.75 ± 3.75 g (Table 2). There were no any significant differences among the treatment groups ($p > 0.05$). During the 150 days culture period, there was a weight gain of oyster of 26.55 ± 0.26 g and 23.74 ± 2.6 g in T2 and T3 respectively. Over the same period sea cucumber in T1 and T2 had respective weight gain of 146.7 ± 6 g and 153.39 ± 2.04 g. Results in the graphical plot indicate that harvesting weight gain of the fed species (Nile tilapia) was highest in T2 and lowest in the control (Figure 2).

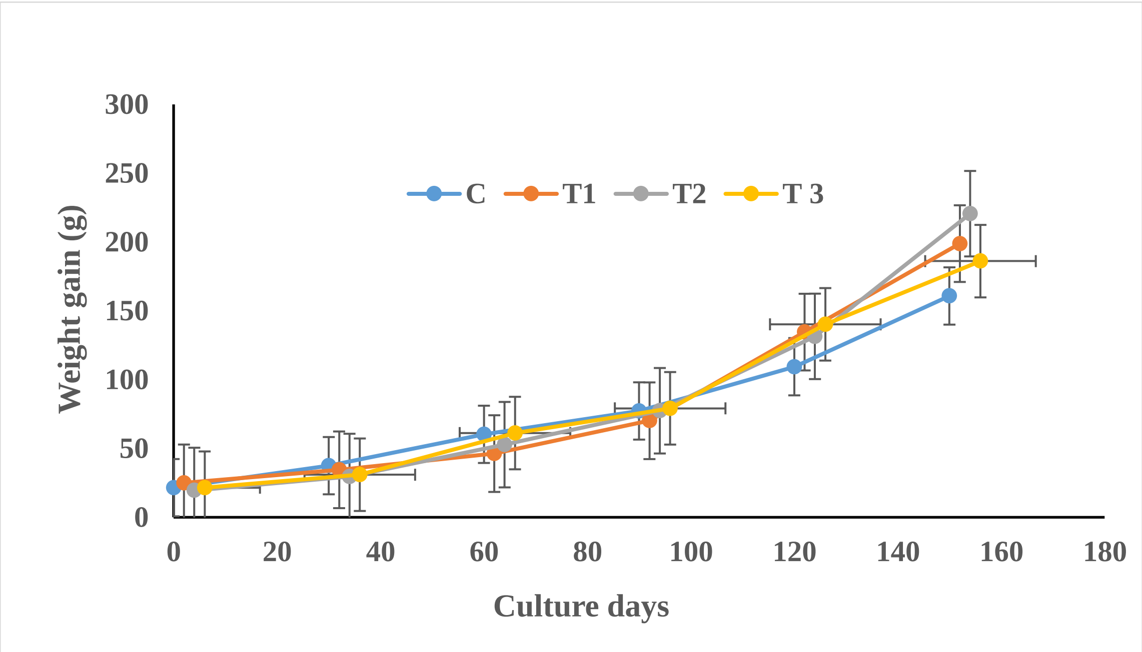

Figure 2. Weight gain in (g) of the fed species (*O. niloticus*) in the four treatments. Values are means (± SEM) of pooled data of replicates in each pond per sampling.

The highest survival was recorded among the Nile tilapia in T2 at 92.9 % compared to those in other treatments which ranged from 79.16 % to 85.40 % in T3 and the control respectively. Survival of oyster was 87.5 % and 79.2 % for the T2 and T3 treatments respectively. Lower survival was recorded for sea cucumber at 53.2 % and 45.6 % for T1 and T2 treatments respectively. Food conversion ratio determined for the fed species, Nile tilapia, was lowest in T2 at 1.78 ± 0.04 and highest in T3 at 2.32 ± 0.3. Mean food conversion ratio was significantly different ($t = -13.67$, $p = 0.046$) for Nile tilapia in the control but not for those in the other treatments.

Results in Table 2 show that the growth of experimental organisms was highest in IMTA treatment T2 which had a combination of Nile tilapia, sea cucumber, and oysters. The fed component, *O. niloticus* in T2 had a weight gain of 182.11 ± 16.6 g, which was followed by T1 that had 173.85 ± 2.05 g and then T3 that had 164.62 ± 6.9 g. The fed species (*O. niloticus*) in the control had the lowest mean weight gain of 136.5 ± 2.8 g. Among the extractive organisms; *H. scabra* in T2 attained higher mean weight gain of 153.39 ± 2.04 g than those in T1 which had 146.7 ± 6 g. *S. cucullata* in T3 had slightly higher weight gain of 26.55 ± 0.26 g as compared to T2 that had a weight gain of 23.74 ± 2.6 g. The daily growth rate was high for *H. scabra* and *O. niloticus* in T2 as compared to those in T1 and control. Specific growth rate (SGR) was lowest for oysters, 4.1 % in both T2 and T3. Figure 3 shows the growth of the organisms in treatment 2 from stocking to harvesting that had full IMTA combination. *O. niloticus* and *H. scabra* performed better as compared to *S. cucullata.*

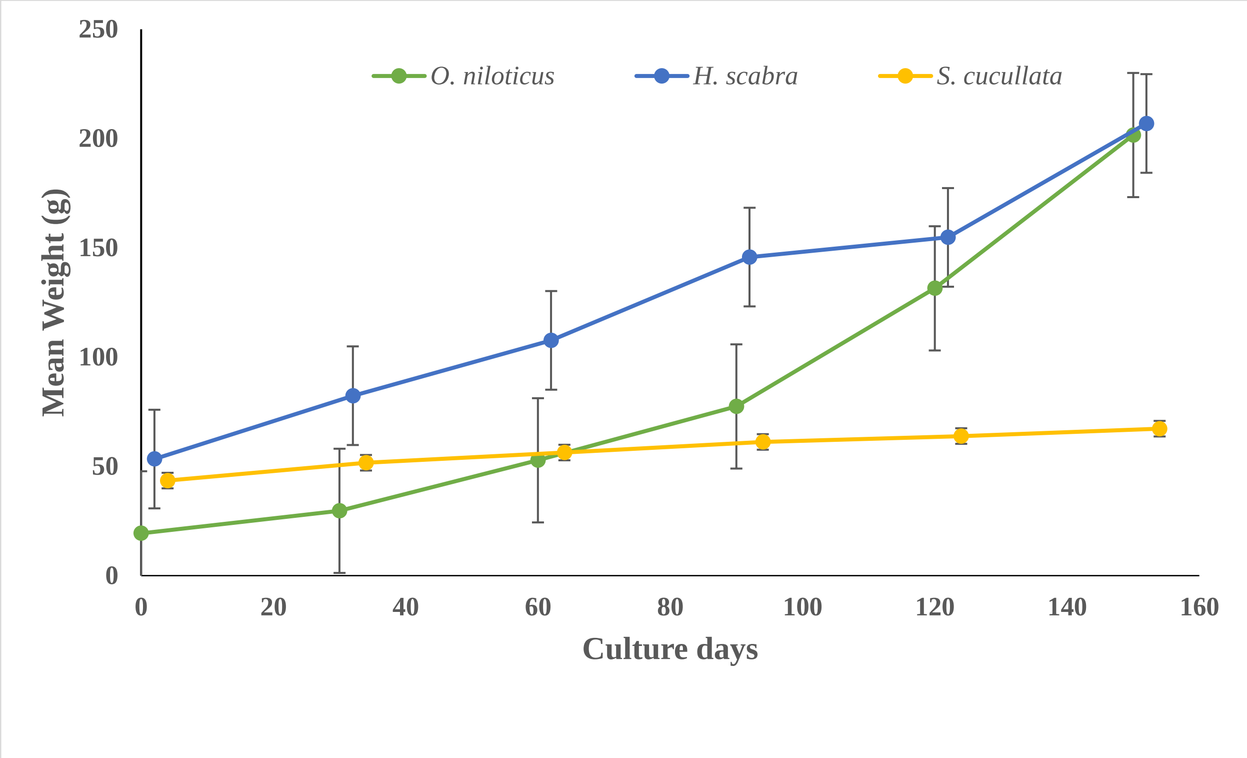

Figure 3. Growth of organisms in full IMTA treatment (T2) from stocking to harvesting.

Water quality parameters

Among the physico-chemical parameters of culture water from the experimental ponds, transparency differed significantly ($p < 0.05$) between treatments being higher (32.72 ± 0.25) in T2 than in the control that had a transparency of 24.84 ± 0.2 (Table 3). Over the 150 days study period, water temperature in the culture facilities varied between 21.4 and 32.6 °C, while salinity ranged from 28.5 to 40.5 ppt throughout the experimental period.

Dissolved oxygen levels were within suitable ranges for culture and was significantly higher ($p < 0.05$) in the IMTA treatments as compared to the control. T2 had the highest dissolved oxygen at 3.901 ± 0.01 mg/l^{-1} whereas the control had the lowest at 3.25 ± 0.01 mg/l^{-1}. The pH values did not vary much between the treatments, ranging from 7.6 in T2 to 8.0 in T3. Both T1 and the control had a pH 7.9. The lowest value of TAN (ammonia nitrogen) was observed in T2 0.024 ± 0.007 mg/l^{-1} which was significantly different ($p < 0.05$) from that of T3 0.076 ± 0.007 mg/l^{-1} but not from T1 and control ($p > 0.05$). The Nitrate–nitrogen differed significantly ($p < 0.05$) being lower in the IMTA treatment T2 (0.025 ± 0.007) mg/l^{-1} which had the three species than in the control (0.057 ± 0.007 mg/l^{-1}) that had tilapia monoculture. Mean value of nitrite- nitrogen was lowest in T2 (0.023 ± 0.004 mg/l^{-1}). There were no any significant differences on phosphate phosphorous concentrations among the treatments.

Economic performance evaluation

Results of the economic analysis showed that, net income, and CBR were significantly better ($p < 0.05$) in T2, T1, and C as shown in Table 4. T1 and T2 had similar CBR which was higher than the control ($p < 0.05$). Net income was highest in the IMTA T2 with a value of USD 77.5 followed by IMTA T1 that had a value of USD 33.9. Higher production and better selling price of the harvested organisms in T2 and T1 contributed to higher profit in IMTA system.

Table 2. Results of analysis of growth parameters of *O. niloticus*, *H. scabra* and *S. cucullata* in the control (C) and the IMTA treatments (T1, T2, and T3).

Treatments	**C**	**T1**		**T2**			**T3**	
Experimental organisms	***O.niloticus***	***O.niloticus***	***H.scabra***	***O.niloticus***	***H.scabra***	***S.cucullata***	***O.niloticus***	***S.cucullata***
Growth and yield parameters								
Stocking density (Ind/m2)	2	2	1.9	2	1.9	2.1	2	2.1
Total Stock (n)	240	240	228	240	228	252	240	252
Initial ABW (g)	21.5 ± 1	25±0.5	53.5 ± 2.5	19.5 ± 1	53.5 ± 1	43.5 ± 1.5	21.5 ± 1	41.5 ± 1
Final ABW (g)	160.8 ± 3.8	198.85 ± 2.5	199.7 ± 3.5	218.81 ± 1.54	206.86 ± 3.04	68.4 ± 0.07	186.12 ±7.9	65.96 ±0.75
Initial stocking length (cm)	13.97 ± 1.5	12.66 ± 0.85	14.41 ± 1.66	13.08 ± 0.63	7.93 ± 0.42	8.94 ± 0.11	8.26 ± 0.41	7.69 ± 0.15
Final stocking Length (cm)	21.07 ± 0.48	20.35 ± 0.14	21.22 ± 1.01	22.19 ± 0.72	15.44 ± 0.78	14.55 ± 0.13	14.32 ± 0.35	14.07 ± 0.11
Individual net weight gain (g)	136.5 ± 2.8	173.85 ± 2.05	146.7 ± 6	182.11 ± 16.6	153.39 ± 2.04	26.55 ± 0.26	164.62 ± 6.9	23.74 ± 2.6
AFCR	2.09 ± 0.3	1.97 ± 0.09		1.78 ± 0.04			2.32 ± 0.3	
Survival % day	85.4	81.25	53.26	92.91	45.6	87.5	79.16	79.2
Daily growth rate (g day-1)	0.91 ± 0.18	1.15 ± 0.01	0.97 ± 0.04	1.21 ± 0.11	1.02 ± 0.01	0.15 ± 0.01	1.09 ± 0.05	0.17 ±0.001
Specific growth rate (% day-1)	5.04 ± 0.23	5.28 ± 0.012	5.28 ±0.017	5.29 ± 0.78	5.31 ± 0.01	4.17 ±0.02	5.21 ± 0.043	4.18 ±0.011
Production (Kg/treatment 150 days-1)	33.78 ± 1.6	38.80 ±2.4	24.47 ± 0.9	45.03 ±4.5	21.75 ± 2.3	14.63 ± 1.08	35.40 ± 2.4	13.44 ± 0.32

Table 3. Mean (±SEM) values of various water quality parameters of pond water collected at 30 day interval from the monoculture (C) and IMTA treatment ponds (T1, T2, and T3).

Water quality parameters

Parameter	**Treatments**				**Sampling time**				
	C	**T1**	**T2**	**T3**	**2/20/2021**	**3/26/2021**	**4/25/2021**	**5/24/2021**	**6/25/2021**
Dissolved oxygen (mgl^{-1})	3.25± 0.011	3.32 ± 0.01[c]	3.901 ± 0.01[a]	3.51± 0.01[b]	3.53 ± 0.01	3.26 ± 0.25[a]	3.32 ± 0.11	3.59 ± 0.067	3.38 ± 0.06
Temperature (°C)	29.37 ± 0.309	28.28 ± 0.3	29.22 ± 0.31	28.91 ± 0.31	27.32 ± 0.27[c]	29.22 ± 0.16[b]	32.15 ± 0.56[a]	32.62 ± 0.23[a]	21.39 ± 0.34[d]
Turbidity (NTU)	28.2 ± 1.7[a]	28.4 ± 1.78[a]	22.3 ±1.7[b]	17.9 ± 1.78[c]	20.11 ± 1.14	20.55 ± 1.21	21.91 ± 0.58	22.73 ± 0.76[b]	23.23 ± 0.95 [a]
Salinity (ppt)	36.95 ± 0.3	35.81 ± 0.31	36.57 ± 0.32	34.81 ± 0.3	36.53 ± 0.55	40.47 ± 0.49[a]	37.86 ± 0.51	36.8 ± 0.27	28.51 ± 0.45 [b]
pH	7.9 ± 0.12	7.93 ± 0.1	7.76 ± 0.13	8.06 ± 0.12	8.02 ± 0.26[a]	7.6 ± 0.034[b]	7.64 ±0.16[b]	8.2 ± 0.11[a]	8.1 ± 0.75[a]
Transparency	24.84 ± 0.2	25.34± 0.25[b]	32.71 ± 0.25[a]	30.56 ± 0.2	22.35 ± 0.39	29.78 ± 0.79[a]	25.49 ± 0.58	25.49 ± 0.58	25.2 ± 0.36
Phosphate phosphorous (mgl^{-1})	0.157 ± 0.04	0.142±0.047	0.053 ± 0.05	0.057 ± 0.04	0.076 ± 0.005	0.277 ± 0.15	0.057± 0.016	0.056± 0.013	0.045± 0.013
Nitrate-nitrogen (mgl^{-1})	0.057± .007[a]	0.052±0.007	0.025 ± 0.007[b]	0.048± 0.007	0.059± 0.005[a]	0.051 ± 0.003	0.042± 0.004	0.045 ± 0.01	0.031±0.005[b]
Ammonia nitrogen (mgl^{-1})	0.067 ± 0.007	0.043±0.007	0.024 ± 0.007[b]	0.076± .007[a]	0.052 ± 0.002	0.06 ± 0.005	0.053± 0.004	0.048± 0.004	0.05 ± 0.007
Nitrite nitogen (mgl^{-1})	0.041 ± 0.004	0.036± .004	0.023 ± 0.004[a]	0.034± 0.004	0.029±0.006[b]	0.028 ± 0.004	0.037±0.003[a]	0.041± 0.003	0.033± 0.002

Means with different superscripts in a row differ significantly ($p < 0.05$).

Table 4. Operational costs and economic returns among monoculture (C) and IMTA treatments (T1, T2, and T3). Calculations were for 150 days experimental duration. Currency indicated in USD (1USD=Ksh 105). (TI is Total Income, TE is Total Expenditure, and NI is Net Income)

Items	Quantity	Price Rate (USD)	C	T1	T2	T3
Operational costs						
Oreochromis niloticus seed	240	0.19	45.7	45.7	45.7	45.7
Holothuria scabra seed	228	0.28		63.84	63.84	
Sacostrea cucullata seed	252	0.14			35.28	35.28
Lime (CaCO3) (kg)	120	0.21	25.1	25.1	25.1	25.1
Fertilizer (kg)	DAP 4.8	0.62	3.01	3	3	3
	UREA 7.2	0.51	3.7	3.7	3.7	3.7
Feeds (kg)	117.5, 88.8, 106, 107	1	118	88.8	106	107
Pond hire	2	28.5	57	57	57	57
Casual labour	5	19.04	95	95	95	95
Total operational Cost			347.5	382.14	434.7	372
Economic returns						
O. niloticus advanced sale (fingerling)	300,220,180,200	0.19	57	42	34.2	38
Sale of harvested *O. niloticus* (kg)	34,38.8,45,35.4	2.4	81	93	108	84.96
H. scabra sale (kg)	24.4, 21.7	19.04		465	413.16	
S. cucullata sale	13.4,14.6	3.8			50.92	55.6
Gross return			138	600	606.28	177
Net income (TI-TE)			-209	217.8^{b}	171.3^{a}	-193
Cost Benefit Ratio (CBR) TI/TE			0.4^{b}	1.57^{a}	1.39^{a}	0.47
Return on Investment (%)	NI/TE*100		-60.2	56.7	39.4	-52.1

Means with different superscripts in a row differ significantly ($p < 0.05$).

Treatment T3 and the control had the lowest income which did not breakeven with USD values of (193) and (209) respectively. The calculated CBR was 1 for both IMTA T1 and T2, a zero value for T3 and 0.4 for the control. The determined return on investment was highest in T2 at 17.6 % while IMTA T1 had 8.6 %. IMTA T3 and the control showed negative rates.

Discussion

Growth performance of the cultured organisms

The comparison of IMTA system and the control made in this study tested the feasibility of culturing fin fish, oysters, and sea cucumbers together in an intertidal earthen pond ecosystem. The results showed that organisms cultured in the IMTA set up realized better growth and production than those in the control monoculture system. Individual weight gain and total production differed among cultured species in the different treatments which concurs with studies by Cunha et al, (2019). Nile tilapia which was the fed component of the IMTA set up in the study showed good performance during the 5 months culture period compared to the co-cultured extractive species; sea cucumbers and oysters.

The low production of sea cucumber observed among the different treatments could be attributed to predation from the Nile tilapia, the fed component in the IMTA set up. Sea cucumbers are deposit feeders that consume mud particles (Tresnati et al., 2019), a feeding habit that helps reduce the organic load from the bottom of earthen ponds while being integrated with other organisms (Funge-Smith and Briggs, 1998). This attribute, coupled with the species high market value makes sea cucumber a good candidate for multitrophic aquaculture systems (Magondu et al., 2021).

In the present study, the fed component of the IMTA T2 showed significantly ($p > 0.05$) high individual body weight of Nile tilapia at 218.82 ± 1.6 g, which was followed by T1 and T3 that had an integration of tilapia and sea cucumber at 198.9 ± 2.6 g and tilapia and oysters at 186 ± 7.9 g respectively. Nile tilapia in the control achieved relatively lower individual weight of 160.75 ± 3.8 g. The better growth of Nile tilapia in IMTA could be attributed to efficient removal of nutrient wastes from the culture environment as nutrient waste, fecal waste and uneaten feeds were extracted from the system and utilized by the extractive organisms such as filter feeders and deposit feeders (Samocha et al., 2015).

The specific growth rate of cultured organism ranged from 4.18 % day^{-1} among oysters to 5.3 % day^{-1} among sea cucumbers. Given the water quality parameters were within optimal range, these findings indicate the conditions for growth and species combinations tested the three IMTA treatments in this trial were favorable. In aquaculture, feed conversion ratio (FCR) is used to determine efficiency of utilization of fed feed that is converted to flesh. Nile tilapia in IMTA treatment T2 in this study had an FCR of 1.78 ± 0.04 compared to T1, T3 and the control which had slightly higher levels Table 2. This might have been as a result of better feed utilization by the cultured organisms. Waite et al. (2014) showed that FCR is related to environmental performance as it provides indications of nutrients loss to the environment and any undesirable output. A lower FCR shows an efficient system (Hasan and Soto, 2017). A similar study reported that 'species combination in an IMTA reduced FCR by 12-15% compared to monoculture' (Shpigel et al., 2016).

Bivalves have been used in IMTA systems based on their potential to directly filter organic particulates from the culture site and also nutrient extraction from the system (Chopin, 2013). Being suspension feeders, bivalves alter the plankton structure which promotes nutrient cycling in the culture environment (Smyth et al., 2013; Murphy et al., 2016). In this study oysters were used as filter feeders and contributed to increased biomass and efficiency of the system. In the current study, integration of oysters was found to be feasible in the co-culture IMTA pond system with Nile tilapia through improving water quality and increasing production in tilapia and sea cucumbers integration. Several studies have shown that extractive species in IMTA systems grow faster than in monoculture which implies that IMTA farms can provide greater economic gains and environmental benefits (Whitmarsh, et al., 2006; Sara et al., 2009; Sanderson et al., 2012).

Survival of culture organisms in different treatments

IMTA treatment (T2) achieved the highest survival for the fed species at 92.2%. Results of water quality and the culture environment in T2 were suitable for culture of the different organisms. Integration led to pond ecological balance that ensured high survival of tilapia as the fed organisms and sufficient nutrients that were captured by the oyster filter feeders which also showed high survival rates of 87.5 % and 79.2 % in T2 and T3 respectively Table 2. Sea cucumber was used as one of the extractive organisms due to its bio-mitigation ability and potential to reduce excess accumulation of waste from the pond bottom (Purcell, 2015; Cubillo et al., 2016). In this study survival of sea cucumber was lowest in treatment T2 that had 45.6 % and T1 that had 53.26 %. Some sea cucumbers were observed with injuries which were likely caused by predatory attacks by Nile tilapia, and it is possible that severe wounds may as well explain the higher overall mortality of this species reported in this study. Incidences of predator attack on *H. scabra* has earlier been reported in studies by Lavitra et al. (2009) and Tresnati et al. (2019). In sea cucumber farming, challenges related to biotic and abiotic parameters are normally experienced (Purcell, 2004; Wang et al., 2004). These include; salinity drops against the optimum of 28 to 31 ppt, Xilin (2004) that occurs in seasons of heavy rainfall (Chen, 2004) and especially during low tides a situation that makes the organisms to burrow underground even when they are supposed to be active and foraging for food. Abnormal occurrence of high numbers of parasites such as Isopods in ponds during hot season has been a cause for sea cucumber mortality (Pitt and Duy, 2004). In addition, abundance of crabs near the culture environments has been blamed for attack on both juveniles and adult sea cucumbers and cause of high mortality (Magondu et al., 2021). Skin ulcerations were also evident which were followed by mucus secretions on the body, skin discoloration and behavior changes as reported by Purcell and Eeckhaut (2005).

The observed mortality of sea cucumber in T1 and T2 could be attributed to the presence of Nile tilapia. These attacks were more serious during foraging for feeds from the pond environment. Nile Tilapia has been categorized as herbivorous fishes (Getachew and Fernando, 1989), which mainly feed on algae, while other workers have classified them as omnivores with food composition dominated by zooplanktons like copepods and rotifers (Bwanika et al., 2004). Such feeding habits may also have contributed to the observed attacks on sea cucumber as the tilapia forage for food at the pond bottom where the sea cucumbers dwell. Dorsal and ventral injuries and skin ulcerations were observed on most injured sea cucumbers. `Other possible predators were mud crabs and mangrove snails which were found to be present in some of the experimental ponds concurring with findings by Tresnati et al. (2019). However, these were removed physically during the pond preparation before stocking and by use of lime to kill any predator eggs and larvae present. Another plausible explanation for the low number of sea cucumber harvested is that some individuals burrowed into the substrate to avoid predator attack by tilapia (Pitt and Duy, 2005). Such burrowing caused substrate disturbance (bioturbation) leading to better nutrient recycling and utilization benefiting the overall culture environment.

Water quality

The water quality parameters in this study showed better results in the IMTA system as compared to the control monoculture. The parameters were stable throughout the experimental period and within acceptable range of finfish mariculture (Biswas et al., 2019). The dissolved oxygen levels shown in the three IMTA treatments could have been attributed to the species combinations that filtered the culture water by consuming the algae available making the culture water less turbid. Presence of fish, oysters, sea cucumber, and phytoplankton contributed to pond ecological balance due to the synergies created. The enhanced water quality led to better fish performance and higher biomass production. Further, less amount of energy was needed to maintain pond oxygen levels which improved the system efficiency. The photosynthetic activity within the systems was dependent on the type of treatment whereby ponds without oysters (T1) and (C) showed significantly lower dissolved oxygen production in comparison with the treatments that had oysters (T2 and T3). The treatments without oysters had lower Secchi disk readings which implied higher turbidity due to suspended particulate matter in the water column which prevented light penetration that hampered photosynthetic activity and consequently feed accessibility. In other studies, poor water quality has often been blamed for lack of sustainability and profitability in aquaculture enterprises (Biao, 2007; Cao et al., 2007). Studies by Newell (1988) showed that oysters filter water at a rate of $0.12m^3g^{-1}$ dry weight per day which has shown potential to extract large amounts of organic particles from the culture water. There were lower nutrient levels in terms of nitrates, nitrites and ammonia in the treatments that had sea cucumber as they prevented formation of anaerobic conditions by consuming high amounts of organic matter in the sediment that caused reduction in ammonia levels hence accelerating nitrification and denitrification processes which improved water quality in the ponds (Uthicke, 2001; İşgören-Emiroğlu and Günay, 2007).

Economic performance

The current study found that IMTA had a higher net income, cost benefit ratio and return on investment than either of the monoculture operations. Despite these encouraging results, IMTA is likely to be justifiable for investors only if there is additional profitability (Whitmarsh *et al*., 2006; Ridler and Ridler, 2011). Whitmarsh *et al.* (2006), Ridler *et al. (*2007), and Shi *et al.* (2013) all suggested that higher profitability is possible with IMTA than with monoculture aquaculture farms, ascribing it to higher growth rates of co-cultured extractive IMTA species, the ability to spread some of the IMTA's administrative and operational expenses over a wider range of products (e.g. marketing and sales costs, salaries and wages, utilities), and access to additional income streams.

Clearly, economic sustainability is a key objective to be considered before venturing into commercial aquaculture practices. Hishamunda *et al*. (2014) showed that without economic viability, aquaculture ventures can only continue if subsidized. Higher profitability in IMTA farms has contributed to its more preference to monoculture practices by farmers attributing it to higher growth rates of the co-cultured extractive species, possibilities of spreading costs over a wide range of products in addition to access to different income streams (Whitmarsh *et al*., 2006; Ridler *et al*., 2007b; Shi *et al*., 2013). Presence of co-cultured items in an aquaculture enterprise can contribute to water quality improvement, additional productivity, and profitability of the farm operation (Fantini-Hoag *et al*., 2022).

A comparative economic performance analysis between three IMTA treatments (TI, T2, T3) and a control monoculture (C) made in this study indicated the possibilities of farming a fin fish, oysters and sea cucumber together in brackish water intertidal ponds. One complete (T2) and two partial IMTA treatments (T1 and T3) were tested in this study in comparison to fin fish monoculture. The full IMTA (T2) performed better in terms of production and economic returns as compared to the other treatments. Gross returns, net income, and cost benefit ratio (CBR) showed significant differences with highest values in T2 followed by T1. This was as a result of increased total production and higher selling price of harvested fin fish and sea cucumbers that had better weight gain in T2. In addition, the estimated market price of oysters increased the net income further for T3. The results of economic analysis show the viability of the IMTA system as a suitable farming option. Under the two scenarios, the analysis revealed that the IMTA system was more profitable, looking at the benefits of product diversification, reduction of production risk and reduced cost of inputs. Review studies by Knowler *et al*. (2020) showed that economics of IMTA has been mainly focusing on financial analysis on profitability, economics of environmental externalities and market analysis on consumer perceptions and acceptability of IMTA systems and products.

Conclusions

The IMTA set up involves maintaining a clean culture environment at reduced costs of inputs such as feed and fertilizers to facilitate improved efficiency while supporting sustainable production. Growth performance of Nile tilapia (the fed species) was better in the IMTA treatments than in the monoculture (control). The co-cultured species sea cucumber and oysters also exhibited good growth in the IMTA ponds. In the present study, Nile tilapia as the fed component of the system contributed to the overall production of the system in addition to providing food and energy that the co-cultured organisms depended upon. The production costs, net incomes, CBR and ROI were higher in IMTA setups. This indicated IMTA production system is a profitable business. The species combination and pond environment were important factors for increasing production suggesting more attention should be paid to these parameters for more economic gains. Sea cucumbers experienced predation attacks from the tilapia leading to their low survival rates. This finding thus recommends further analysis of the Nile tilapia stomach contents. Therefore, the findings of this study revealed that use of Nile tilapia as fed species in an IMTA system with sea cucumbers as an extractive component is not viable due to predation attacks. Possibilities of using other fed species for IMTA studies need to be explored and provision of appropriate substrates that the sea cucumbers can burrow in to escape predation.

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Ethical approval

The author declares that this study complies with research and publication ethics

Informed consent

Not available

Conflicts of interest

There is no conflict of interests for publishing their study

Data availability statement

The authors declare that data are available from authors upon reasonable request.

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Author contribution

The experiment was conceived by Author 1 - as part of the requirements for a PhD in Fisheries degree of Pwani University.

Author 1: Writing original draft, Conceptualization, Data curation, Formal analysis, sample preparations, chemical analyses, data analysis and drafted the manuscript.

Author 2 Supervision, Validation, Visualization, Project administration, Review, Editing and approved the final version of the manuscript for submission.

Author 3 Supervision, Visualization, Review, Editing, and approved the final version of the manuscript for submission.

Author 4 Supervision, Validation, Visualization, Project administration, Review, Editing critical examination, and approved the final version of the manuscript for submission.

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