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Cover image: The mudskipper *Periophthalmus argentilineatus* on the mangrove of Ulo Beach, Cabo Delgado, North Mozambique. © José Paula, 2006

Effects of different types of manure on the culture of marine plankton as a potential source of food for mariculture hatcheries

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The study examined different types of organic manure on the culture of marine plankton as a potential source of food for rabbitfish, *Siganus stellatus*, larvae. Cow dung showed significantly higher species abundance and diversity of cultured marine plankton followed by chicken and finally mixed media manure ($p<0.05$). A total of 36 genera of phytoplankton (21) and zooplankton (15) were identified in all culture media. Class Bacillariophyta was the most abundant and diverse group which accounted for 41.3 % of the total phytoplankton. Calanoida was the dominant group of the identified zooplankton, accounting for 51.7 %. It was observed that the organic manure used favoured the growth of commercially important species of phytoplankton such as *Chaetoceros* sp., *Skeletonema* sp., *Chlorella* sp., *Isochrysis* sp., *Nannochloropsis* sp. and *Spirulina* sp., and zooplankton such as *Eurytemora* sp., *Calanus* sp., *Oithona* sp., *Branchionus* sp., *Moina* sp. and ostracods. The growth performance and survival rate of early stage rabbitfish larvae fed with live marine zooplankton performed better compared to those fed with *Artemia* spp. and commercial dry feed. This indicates that zooplankton have the potential to enhance growth performance and survival rate, hence increasing productivity and the development of mariculture.

Keywords: marine plankton, organic manure, cow dung, chicken droppings, mariculture

Introduction

There is continued and growing demand for alternative and proper nutritive food for commercial marine finfish and shellfish species, especially during larval stages in captivity (Evjemo *et al.,* 2003; Iyiola, 2014; Delbos, 2019). Marine zooplankton are appropriate food for them due to their nutritious quality, and the newly hatched nauplii are rich in essential fatty acids (EFA), polyunsaturated fatty acids (PUFA), docosahexaenoic acid (DHA), and omega-3 fatty acids in the most adequate ratios (Delbos, 2019). Other common food (Rotifers and *Artemia* sp.) are deficient in highly unsaturated fatty acids (HUFA) that are essential for better growth performance and survival rate of marine fish larvae (Evjemo *et al.,* 2003; Delbos, 2019). There are various technologies developed worldwide to culture plankton, especially microalgae and a few species of zooplankton such as rotifers, *Atermia* spp., and copepods (Creswell, 2010; Dhont *et al.,* 2013; Delbos, 2019; Mtaki *et al.,* 2021). Most of these advanced technologies have been used for freshwater phytoplankton and zooplankton while there is limited information for marine plankton culture for coastal marine fish hatcheries. These efforts have also not considered small fish farmers who would struggle to afford the technology and materials for further development and improved production. Furthermore, of marine fish nursery production and commercial

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hatcheries in coastal areas are not fully integrated with the production of live food in large commercial facilities to meet the demand of small fish farmers.

Mariculture hatcheries in coastal regions are facing challenges with rearing early stages of marine fish species. In addition, commercial and local dry diets are used which are not ideal for better growth and survival rate of larvae and fry stages due to their small size and under developed digestive system. They are unable to provide easily digest dry feed compared to live food which has been shown excellent quality nutrients for marine fish species (Evjemo *et al.,* 2003; Olurin *et al*., 2012, Delbos, 2019). Commercial *Artemia* spp. and other genera of calanoid, harpacticoid and cyclopoid have been reported as ideal live feed in several geographic areas (Dhont *et al.,* 2013; Delbos, 2019). Despite these positive findings, rotifers and *Artemia* continue to be the live feeds of choice in commercial hatcheries, because copepods are not currently cultured at sufficient densities to be economically efficient on a commercial scale (Dhont *et al.,* 2013). Furthermore, there is limited technology for culture especially using saltwater for marine hatcheries. Also, the use of single species for fish culture which is most practiced is not recommended because mixed species of live food are more nutritious than using single species. There is a need to provide a nutritive variety of live food to larvae and fry stages, hence improving their growth, survival rate, and immune system to resist diseases. Furthermore, the collection and use of plankton directly from the wild is not advisable due to susceptibility to pathogens and parasites. Because of this, the plankton should first be obtained from the wild as seed, isolated and pure strains cultured as potential live food in the hatchery to avoid infections from the wild and harmful toxin-producing species.

Previous studies have reported that plankton culture and its production depends on the type of manure used (whether organic or inorganic) to facilitate their growth in the water medium (Wurtz, 2009; Abu Hena and Hishamuddin, 2012). A wide variety of organic materials have been used to promote the growth of plankton as well as stimulate the development of invertebrates and other micro-organisms from aquatic systems (Iyiola, 2014). Manures from chickens, goats, rabbits, sheep, cattle and horses have been reported as excellent fertilizers for culturing plankton (Iyiola, 2014; Wurtz, 2009). Most of these studies were based on freshwater plankton and there is limited information for marine plankton culture. The present study was conducted to evaluate the effect of using different types of manure on plankton abundance, to conduct a qualitative and quantitative analysis of cultured marine phytoplankton and zooplankton, to determine nutrient of media culture used, and the potential of live marine zooplankton as a source of food for rabbitfish *S. stellatus* larvae. Furthermore, environmental parameters for culturing marine plankton were determined.

Materials and methods

Collection of manure and culture media preparation

Cow dung and chicken droppings were collected from the local farming villages of Muungoni, Bambi and Muyuni in Unguja Island, Zanzibar as culture media for marine plankton. Precaution was taken to ensure that the manure was not contaminated with treatments that are sometimes used for livestock to control flies and which may inhibit the growth of some zooplankton, especially cladocerans (Oladele and Omitogun, 2016). The manure was transported in dry, clean zip bags to the wet laboratory at the Institute of Marine Sciences, University of Dar es Salaam. The manure was allowed to dry in sunlight because moisture content can affect manure quality (Bocek and Gray, 2002). According to Oladele and Omitogun (2016) the recommended dosage rate for culture using organic manure is 1.5 g/L. For this study 150 g of manure was added in 10 L of salty water to obtain culture media for each treatment. The culture media was allowed to remain in water for 3 days for nutrients to mineralize into the water. Thereafter, filtration was conducted using a 1 mm mesh size net to eliminate unwanted particles and organisms, followed by autoclaving using an HV-85 Top-Loading 85L Autoclave (Amerrex Instruments, Inc. United States) at a temperature range 105-135 °C. The culture media were then allowed to cool and inoculated into each polyethylene bag culture system treatment (cow dung, chicken droppings and mixed, separately in replicates) and left for 4 days with sufficient light (50-watt bulbs) for phytoplankton growth at a room temperature of 27 °C maintained with air conditioners, while each culture system was equipped with an aeration tube for oxygenation.

Sampling procedure and experimental design

The collection of marine phytoplankton and zooplankton was conducted by using nets with mesh sizes of 30 μm and 80 μm respectively, from the wild near the shore of Buyu, Unguja Island, Zanzibar. 50 L of water was concentrated in a phytoplankton net while zooplankton were collected by towing the net behind the boat on the surface at very low speeds for 15 minutes to concentrate the samples for zooplankton culture. The concentrates of phytoplankton and zooplankton were kept alive separately in 10 L plastic containers containing autoclaved sea water aerated with a battery-operated pump, and thereafter placed in the prepared polyethylene culture systems (with volume of 100 dm^3). A total of 24 (12 for phytoplankton and 12 zooplankton) polyethylene bag culture systems were used for marine plankton culture experiment (Fig. 1). Four treatments were used (sea water only as a control; cow dung media; chicken droppings media; and a mixture of cow dung and chicken droppings media) at the ratio of 50:50 by dry weight, respectively. Live concentrated marine plankton was added to each culture treatment (100 dm^3 culture system contained sea water with media culture) in equal amounts of 1 L. All experimental treatments were triplicated.

Nutrient determination of media culture used

500 ml of water from the culture media was prepared by filtering through 0.45 µm millipore filter paper in a filtration pump unit. A SHIMADZU Spectrophotometer UV-1201-Japan was used to determine nitrate and phosphate concentrations according to Parsons *et al.* (1984). Nitrate was determined by taking a 100 ml sample in an Erlenmeyer flask and adding 2 ml of ammonium chloride (NH_4Cl). 5 ml of the mixture was poured onto the top cadmium-copper column and allowed to pass through. The remaining sample was added into the column after which the Erlenmeyer flask was used to collect 50 ml of the sample at the bottom of the collection tube. 1.0 ml of sulphanilamide solution was added in the 50 ml sample using a micro-pipette and allowed to react for 5 minutes. 1.0 ml of naphthyl ethylenediamine solution was then added and the contents mixed immediately. The extinction of a solution in a 10 cm cuvette was measured at 543 nm wavelength. Nitrate was determined using the equation:

µg-at N/l = (corrected extinction x F) – 0.95C ....(1)

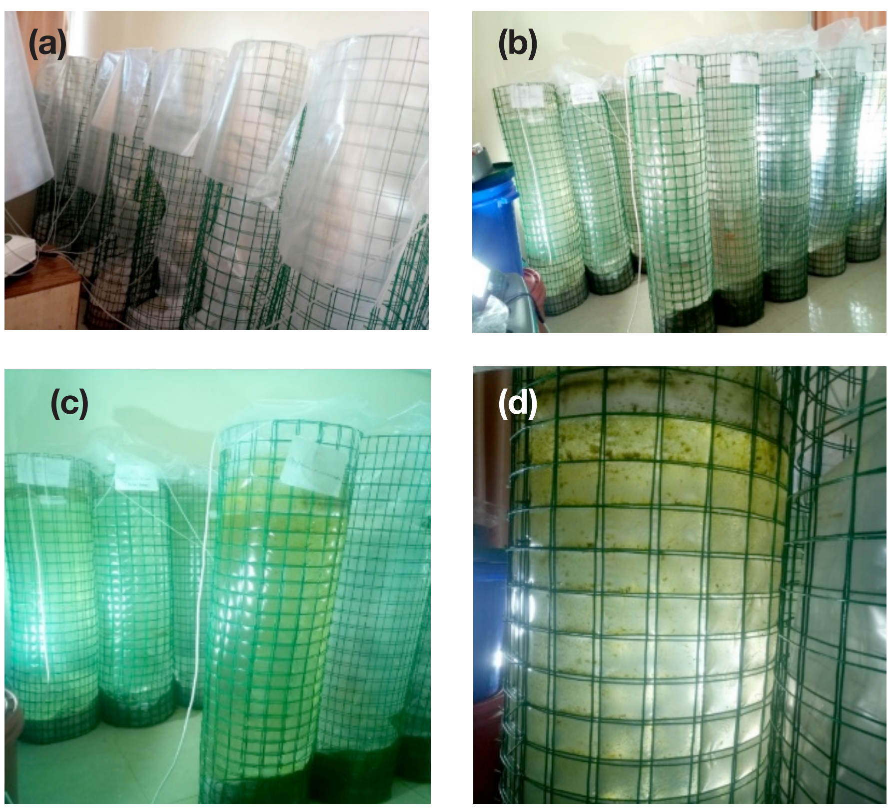

Figure 1. (a) Experimental setup before the polyethylene bags culture system was filled with sea water; (b) Setup after polyethylene bags culture system was filled with sea water and media inoculation; (c) colour changes started to be observed in a third week; and (d) complete colour change (greenish and brown) after sixth week with filamentous algae observed.

Where: C = Concentration of nitrite in the sample in µg-at N/l.

Phosphate was determined by taking a 10 ml sample (in duplicate) and adding 1 ml of mixed reagent (Ammonium molybdate solution, Sulphuric acid, Ascorbic acid and Potassium antimonyl-tartrate at a ratio 2:5:2:1, respectively) using a micropipette and mixed. After 5 minutes the extinction of a solution was measured in a 10-cm cuvette against distilled water at 885 nm wavelength. Phosphate concentration was then determined using the equation:

µg-at P/l = corrected extinction x F............................((2)

Where F is a factor of a standard solution. The standard curves for nitrates and phosphates were determined through serial dilutions of analytical grade Potassium Nitrate (KNO_3) and analytical grade Potassium di-hydrogen phosphate (KH_2PO_4) for nitrate and phosphate respectively.

Qualitative and quantitative analysis of cultured marine plankton

Population abundance and diversity of cultured marine plankton was determined by measuring 3 ml out of a concentrated 20 liters of water from the culture system after stirring thoroughly to ensure even distribution of organisms in the beaker. Identification and counting of specimens in each sample was conducted by using a Sedgewick-rafter cell and inverted microscope using the guide provided by Conway *et al.* (2003). The phytoplankton cells were identified using a guide by Bryceson (1977).

Feeding and sampling of rabbitfish larvae for comparison of growth and survival rate

A total of 270 larvae and fry (with an average weight of 0.25 g and length of 1.32 cm) of *S. stellatus* were collected from Chwaka Bay in Unguja Island using a trap and seine nets. Nine aquaria (dimension of 3600 cm^2 and volume 80 dm^3) were used to examine the growth and survival rate when fed with the live cultured marine zooplankton *Artemia* spp. and a commercial dry feed. All experimental setups were triplicated. Commercial dry powder feed (35 % crude proteins) was bought from Eden Agri-Fish Farm Limited together with *Artemia* spp. cysts. One gram of dry cysts was added to 1000 ml of aerated saline water (35 practical salinity units) followed by treatment with sodium hypochloride to decapsulate cysts and left for 24 hours for eggs to hatch (Asem, 2011). Phytoplankton in this study were harvested at an interval of one week after observing clear greenish/brownish colour in the polyethylene culture system. 1.5 L of phytoplankton was used in the zooplankton culture systems to ensure continuous production of zooplankton in each culture system for feeding rabbitfish larvae and fry. Each aquarium had 30 individual fish larvae and fry. Weight differences between them were taken into consideration. Larvae and fry were fed four times a day (9:00 am, 12:00 pm, 3:00 pm and 5:00 pm). Mean weight and length measurements were taken at an interval of 7 days after recording initial weight and length. Length measurements were taken with a transparent ruler (mm/cm) and weight (mg/g) measurements were recorded with a very sensitive electronic balance.

Determination of growth performance indices of S. stellatus, and water quality parameters

Growth performance indices including final body weight (FBW), body weight gain (BWG, %), specific growth rate (SGR, %/day), feed utilization in terms of feed conversion ratio (FCR), and survival rate were measured according to Ridha (2006). In all culture systems and aquaria, water quality parameters such as pH, dissolved oxygen, temperature and salinity were measured twice a day at 10:00 pm and 4:00 pm using a pH meter, DO meter and refractometer, respectively.

Data analysis

All data were tested for normality and homogeneity using Levene's test at a significance level of $p<0.05$. Population abundance, species diversity and similarity of marine plankton cultured with different types of manure were determined with the Shannon-Wienner index of diversity by using Plymouth Routines in Multivariate Ecological Research (PRIMER) 6.1.2 version software. Paleontological Statistics (PAST) 3 version software was used for Analysis of Variance (ANOVA) to compare the means and state the significant differences followed by pairwise-comparison. Constrained ordination analyses of plankton cultured with different media were performed where abundance data were square root transformed to reduce the contribution of most proportion species. The Bray-Curtis similarity matrix was then generated followed by clustering analysis. A complete linkage dendrogram plot and Multidimensional Scaling Map (MDS) was generated. Growth performance, survival rates, means body weight of rabbit fish larvae and fry were compared using ANOVA followed by Turkey post hoc test.

Table 1. Mean values plus the standard error of the mean of water quality throughout the experimental period.

Water quality parameters	Media culture used (Mean value ± SE)			
	Cow dung	Chicken droppings	Mixed media	Sea water only
Temperature (°C)	27.3 ± 0.27	27.0 ± 0.28	26.9 ± 0.28	27.1 ± 0.28
pH	8.54 ± 0.02	8.63 ± 0.09	8.54 ± 0.04	8.63 ± 0.01
Oxygen saturation (%)	97.55 ± 0.21	94.3 ± 4.62	97.9 ± 0.99	94.15 ± 2.61
Dissolved oxygen (mg/l)	6.25 ± 0.19	6.15 ± 0.54	6.54 ± 0.16	6.21 ± 0.15
Salinity (psu)	34.8 ± 0.14	34.65 ± 0.07	34.85 ± 0.35	34.75 ± 0.35

Results

Water quality parameters in marine plankton cultures

In all treatments water temperature ranged from 25.65 °C to 29.05 °C, dissolved oxygen varied from 4.05 to 8.45 mg/L, pH varied from 7.3 to 8.4, and salinity ranged from 33.9-35.8. The mean of all water quality parameters monitored are presented in Table 1. No significant differences were observed among all treatments ($p > 0.05$) in all water quality parameters. All water quality parameters were within the safe range for the growth of phytoplankton and zooplankton.

Nutrient concentration in media culture used

Nutrient analysis of all manure and sea water (not fertilized) was carried out for nitrates and phosphates, and the results are presented in Table 2. There was a significant difference ($p<0.05$) in nutrient concentration in all culture media used, where cow dung had the highest concentrations of phosphates followed by chicken droppings, and mixed media having the lowest. Nitrate concentration was highest in chicken droppings followed by cow dung and mixed media respectively.

Community structure of marine plankton cultured in the different media

The results showed that there was a significant difference among all treatments in both abundance and species diversity of marine plankton ($p<0.05$). The species diversity was significantly higher in the cow dung culture medium followed by chicken droppings and mixed media culture. Poor results were observed in sea water medium culture as a control and all results were positively correlated with nutrient concentration.

A total of 36 genera of marine plankton were morphologically identified in all culture media, with 21 species of phytoplankton belonging to the Bacillariophyta (diatoms), Dinophyta (dinoflagellates), Mediophyceae, Chlorophyta (green algae), Cyanophyta (cyanobacteria) and Dictyochophyceae (Table 7 and Fig. 3). Overall, the class Bacillariophyta was the most abundant and diverse group and accounted for 41.3 % of the total phytoplankton in the culture experiment with 9 species. Cyanophyceae ranked second in terms of abundance (29.9 %) and this group was dominated by 7 species, followed by Mediophyceae (18.5 %). Other classes had less than 10 % and were each represented by single species. The first week of the phytoplankton culture system was dominated by *Guinardia delicatulata, Chaetoceros* sp.*, *Chlorella vulgaris***, Chlorophyta (green algae) and *Eucampia zodiacus*, and were followed by other commercial species after two weeks such as *Nannochloropsis* sp. and *Spirulina subsalsa*** and other filamentous algae.

Table 2. Nutrients concentration (µg. atom/L) of manure used during experimental culture.

Culture media	Nitrate (NO_3^-)	Phosphate $(PO_4)^{3-}$
Cow dung manure	2.1571 ± 0.01	0.6812 ± 0.02
Chicken droppings	2.1648 ± 0.03	0.64645 ± 0.01
Mixed manure	2.0052 ± 0.02	0.58755 ± 0.01
Sea water	0.0543 ± 0.0	0.0632 ± 0.00

Table 3. The presence (X) and absence () of phytoplankton species cultured from different manure.

Phytoplankton (Taxa group)	Species identified	Sea water only	Cow dung manure	Chicken manure	Mixed manure
Class Bacillariophyceae (Diatoms)	*Guinardia delicatulata*	X	X	X	X
	Melosira sp.			X	X
	,		X	X	X
	Leptocylindricus sp.		X	X	X
	Rhizosolenia alata		X	X	X
	Synedra formosa		X	X	X
	*Skeletonema costatum***		X	X	X
	Pleurosigma/Gyrosigma		X	X	X
	Amphisolenia sp.				X
Class Chlorophyceae	*Chlorella vulgaris***			X	X
Class Prymnesiophyceae	*Isochrysis* sp.**	X	X		
	Nannochloropsis sp.**				X
Class Mediophyceae	*Eucampia zodiacus*	X	X	X	X
Class Cyanophyceae	*Schizothrix arenaria*		X	X	X
	*Spirulina subsalsa***		X	X	X
	Johannesbaptistia pellucida			X	X
	Prorocentrum sp.		X	X	X
	Leptocylindricus sp.		X	X	X
	Rhizosolenia alata		X	X	X
	Synedra formosa		X	X	X
Class Dinophyceae	*Phalacroma rotundatum*			X	

**Commercially important species for mariculture development.

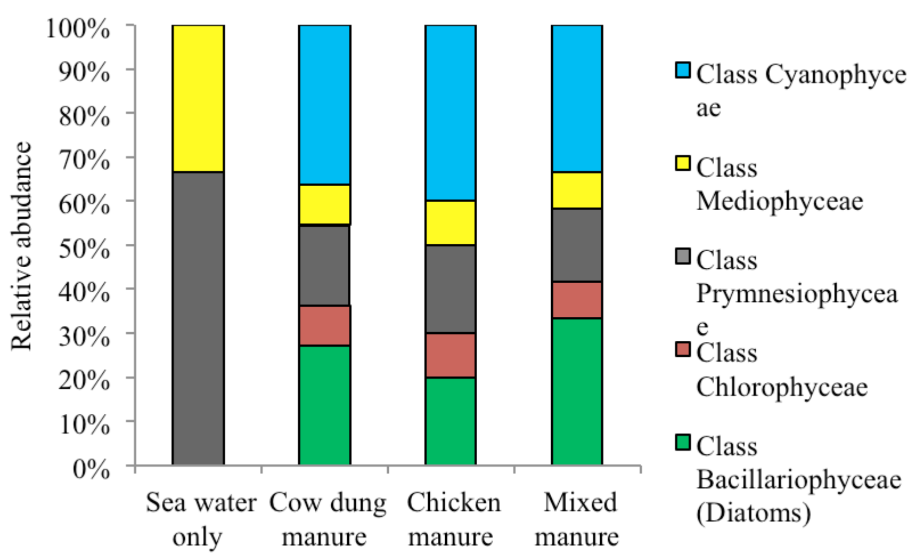

Figure 2. Relative abundance of phytoplankton species in the culture media treatments.

Table 4. The presence (X) and absence () of zooplankton species cultured from different manure.

Zooplankton (Taxa group)	Species identified	Sea water only	Cow dung manure	Chicken manure	Mixed manure
Calanoida	*Eurytemora affinis*		X	X	X
	*Canthocalanus pauper***		X	X	X
	Pseudocalanus sp.		X		X
Cyclopoid	*Oithona helgolandica***		X	X	X
	Centropages furcatus		X		X
Harpaticoda	*Microsetella* sp.		X	X	X
	Corycaeus agilis	X	X	X	X
Euphausiidae	*Paracalanus parvus*		X	X	X
Cirripedia	*Nauplii*	X	X	X	X
Rotifera	*Brachionus* sp.**		X		X
Cladocera	*Daphnia sp. (Moina micrura)* **		X		X
Cnidaria	*Hydroid medusae*		X	X	X
Ostracoda	*Ostracods* sp.**		X		X
Decapoda	*Caridina nilotica***				X

**Commercially important genera/species for mariculture development

Fifteen genera of zooplankton were identified belonging to major groups such as Calanoida, Euphausiidea, Cirripedia, Rotifera, Cyclopoida, Harpaticoida, Cladocera, Ostracoda, Decapoda and Cnidaria (Table 4 and Fig. 3). Of these, the Calanoida was the dominant group which accounted for 51.7 % followed by Cyclopoida (32.6 %) of all zooplankton identified (Fig. 4). In the first week of the zooplankton culture the system was found to be dominated by the calanoid *Canthocalanus pauper*, the cladoceran *Daphnia* sp. and the harpaticoid *Corycaeus* sp.

Cluster analysis and multidimensional scaling (MDS)

Cluster analysis and multidimensional scaling (MDS) were performed to find out the degree of similarity of the species composition of marine plankton. The hierarchical cluster analysis revealed the similar nature between the species composition of the individual culture media. The similarity was observed to be strong between cow dung and chicken manure at about 90 %, followed by 65 % for mixed manure compared with other culture media, while lowest

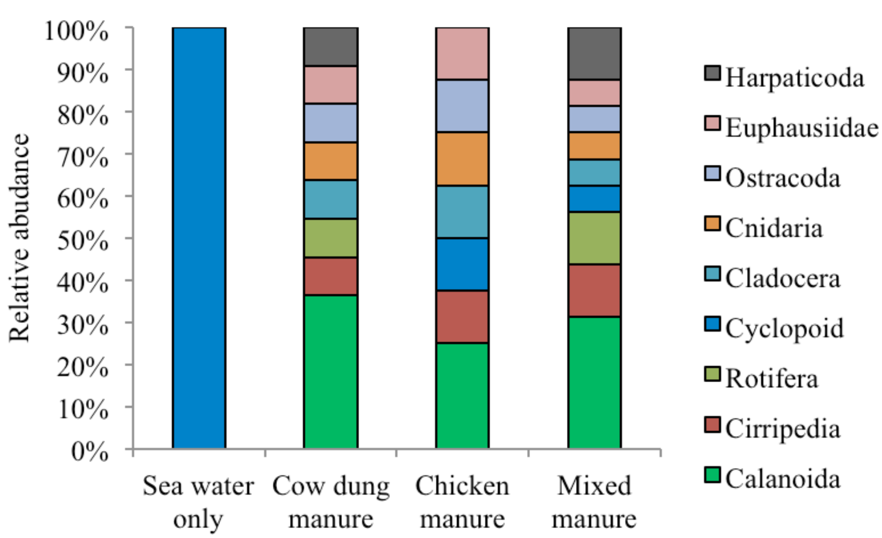

Figure 3. Relative abundance of zooplankton species in the culture media treatments.

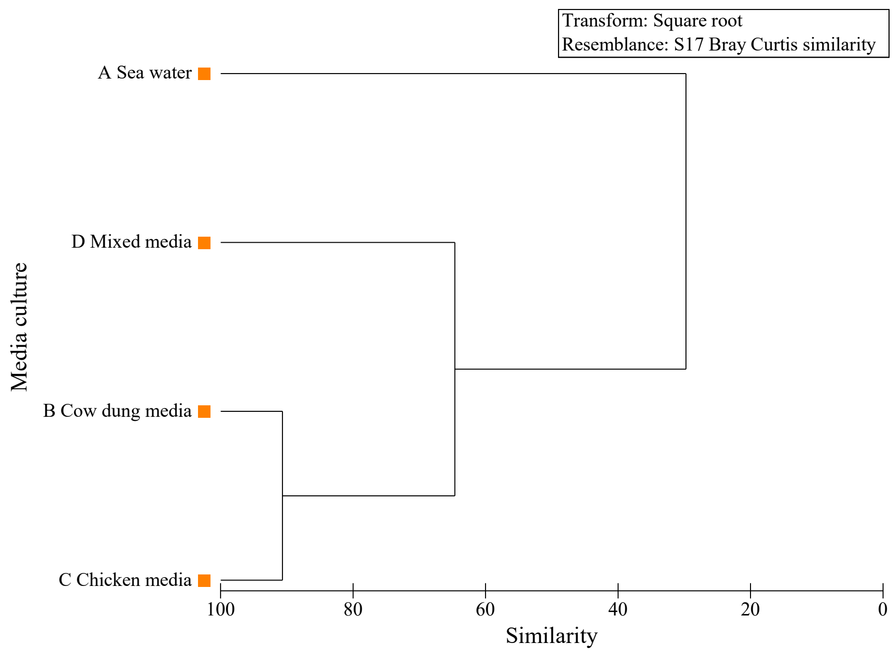

Figure 4. Species diversity percentage similarity of marine plankton (phytoplankton and zooplankton) grown in different manure treatments.

similarity of about 20 % was observed in marine plankton cultured in sea water only (Fig. 4). This was further confirmed by the MDS map (Fig. 5). The stress value was found to be less than 0.01 which is an excellent ordinance pattern that indicates distances among culture media are perfect and are a good representation of the observed data.

Growth performance and survival rate of rabbitfish larvae and fry

There was a significant difference in the growth performance and survival rate of *S. stellatus* larvae and fry fed with live *Artemia* spp. and commercial dry feed ($p<0.05$). The highest growth performance of rabbitfish larvae and fry in terms of final body weight (FBW),

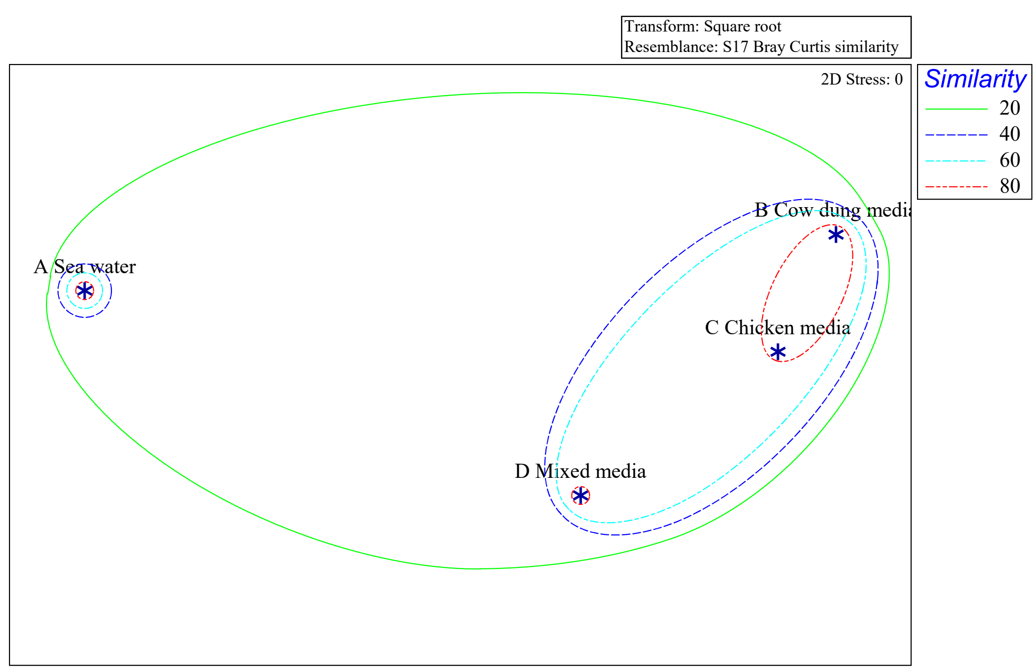

Figure 5. Multidimensional Scaling (MDS) for marine plankton composition of media culture used.

Table 5. The mean values of growth performance parameters of the rabbitfish *S. stellatus* larvae and fry.

Growth parameters	Diet treatments		
	Marine-live zooplankton	*Artemia* spp.	Commercial dry feed
Initial mean body weight (g fish^{-1})	0.22 ± 0.03	0.23 ± 0.01	0.23 ± 0.05
Initial body length (cm)	0.6 ± 0.12	0.5 ± 0.02	0.5 ± 0.04
Final mean body weight (g fish^{-1})	6.05 ± 0.19	5.97 ± 0.23	7.22 ±0.32
Mean body weight gain (g fish^{-1})	5.83 ± 0.21	5.74 ± 0.23	6.99 ± 0.31
Final body length (cm)	2.4 ± 0.11	2.3 ± 0.11	3.9 ± 0.21
Specific growth rate (% day^{-1})	1.806 ± 0.00	1.789 ± 0.00	1.986 ± 0.00
Feed conversion ratio	2.08 ± 0.05	2.26 ± 0.05	1.72 ± 0.03
Survival rate (%)	96 ± 0.00	93 ± 0.00	86 ± 0.00

body weight gain (BWG) and specific growth rate (SGR) was observed in the fish fed with live marine zooplankton compared with other diet treatments ($F(2,444) = 3.12$, $p = 0.04$). This was followed by fish fed with *Artemi*a spp. while poor performance and low survival rate was recorded in the early stages with commercial dry feed. The best feed utilization efficiency of larvae and fry in terms of feed conversion ratio (FCR) was observed in fish fed with live marine zooplankton, followed by those fed with *Artemia* spp.. In both cases, slightly lower growth performance and the feed utilization efficiency was observed in the fish fed commercial dry feed. The survival rate in all treatments varied significantly at $p<0.05$. The mean values of growth performance, feed utilization efficiency, and survival rate of the rabbitfish larvae and fry reared in the 9 aquaria by feeding them 3 experimental diets are summarized in Table 5.

From Figure 6 it can be seen that the live marine zooplankton and *Artemi*a spp. performed better than commercial dry feed at the early stages. However, this trend changed after 21 days when good performance was observed for the fish fed commercial dry feed in terms of survival rate and body weight gain. These results imply that commercial dry feed shows better performance during the grow-out stage of rabbitfish as compared to the early larval stages.

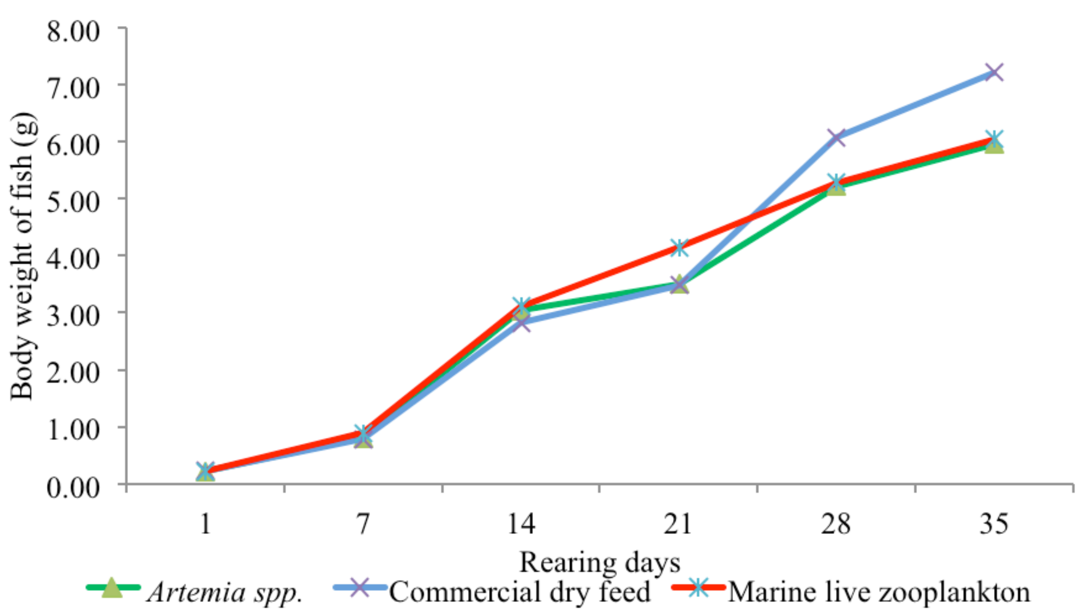

Figure 6. Growth performance of fingerlings of *S. stellatus* fed different types of diets.

Discussion

Water quality parameters in the plankton culture system

The culture of marine plankton (phytoplankton and zooplankton) is affected by different environmental factors such as water quality, including water temperature, salinity, pH, dissolved oxygen, and nutrient (phosphate, PO_4 and nitrates, NO_3) concentration. Zooplankton are totally dependent upon the water to breathe, feed, grow, excrete wastes, maintain salt balance and reproduce (Rottmann *et al.* (2003). However, phytoplankton depend mainly on the amount of nutrients and light available in order to grow. Therefore, ensuring adequate water quality parameters including light penetration is critical to successful culture of phytoplankton and zooplankton for aquaculture production (Olurin and Aderibigbe, 2006). In the current study, water quality parameters monitored included pH, oxygen, temperature and salinity.

All water quality parameters for culturing marine plankton were within the range for survival and growth of plankton. As recommended by Bocek and Gray (2002), the optimum temperature for culture of tropical aquatic organisms is between 25 – 32 °C; pH should range between 6.5 – 9.0, and the recommended level of dissolved oxygen in the culture system must not be less than 5.0 mg/L. The observed temperature values in this study fall within acceptable ranges for culturing plankton as recorded by Rottmann *et al.* (2003). Average dissolved oxygen (DO) was 6.31 ± 0.39 mg/L and the results of the present study are consistent with DeLong *et al*. (2009) and Bocek and Gray (2002), who recommended 5 mg/L as the minimum DO requirements for survival of aquatic organism in captivity. This was achieved through continuous aeration through the aerator pump.

Community structure of marine plankton in relation to nutrient concentration

The growth of phytoplankton depends mostly on the availability of nutrients such as phosphates and nitrates. In turn the phytoplankton becomes a source of food to zooplankton, fish larvae and other larger organisms. In the present study, adequate nutrient concentrations were observed in the cow dung and chicken droppings media, moderate levels in the mixed media, and poor levels in sea water. A linear relationship between nutrient concentration and the amount of phytoplankton and zooplankton was observed. The amount of nutrients in the manures used were slightly different from that reported in Ipinmoroti and Iyiola (2011) where the percentage nitrogen and phosphate in the form of dry weight of was 2.34 % and 0.24 % respectively; perhaps due to different geographical conditions and sources . The least amount of nutrients was recorded in the mixed medium and sea water control. The sea water only control experiment had the lowest concentration of phosphates and nitrates (Table 2) and had poor phytoplankton and zooplankton growth. The control produced low levels of zooplankton because of low algal/ phytoplankton concentrations. This emphasizes that culture systems for raising phytoplankton and zooplankton must be adequately fertilized.

The cow dung culture media accounted for the highest plankton abundance and diversity followed by chicken droppings, and this was largely due to the high nutrient content (both nitrates and phosphates) available as shown in Table 2. This was similar with the research findings of Ipinmoroti and Iyiola (2011), and Wurtz (2009) where high nutrient composition in organic media was also important for the growth of plankton in captivity. However, according to their study based on freshwater zooplankton, poultry (mean mixed manure from chicken, birds and others) showed the best results for the growth of zooplankton. In this study the mixed growth medium had slightly lower nutrient concentrations, possibly due to dilution. A high level of similarity in marine plankton was observed between media with cow dung and chicken droppings, while a low similarity was observed with mixed manure media and sea water only. This might be due to differences in nutrient composition. Iyiola (2014) explained that the two important components of algal nutrients are phosphates and nitrates and both have a linear relationship with the amount and diversity of plankton production. Furthermore, it has been observed that the organic culture media with cow dung, chicken droppings and mixed media favour the growth of commercially important species of marine plankton such as the phytoplankton *Chaetoceros* sp., *Skeletonema* sp., *Chlorella* sp., *Isochrysis* sp., *Nannochloropsis* sp, and *Spirulina* sp., and the zooplankton *Eurytemora* sp., *Calanus* sp., *Oithona* sp., *Branchionus* sp., *Moina* sp. and ostracods, that can be used in mariculture. This study is supported by the results obtained by Creswell (2010), Conceição *et al*. (2010), Iyiola (2014), and Oladele and Omitogun (2016) who reported that some species such as *Chaetoceros* sp., *Skeletonema* sp., *Chlorella* sp., *Isochrysis* sp., *Brachionus* sp., *Daphnia* spp., *Moina* sp. and cyclopods favour manure such as cow dung and chicken droppings and are widely used in hatcheries as startup food for fish larvae and fry stages.

Growth performance and survival rate of rabbitfish larvae and fry

Growth performance, feed utilization efficiency and survival rate are impacted by differences in the quality of the feeds in terms of nutrient composition and other environmental stress factors (Yousif *et al.*, 2005; Ogunji *et al.*, 2008). In this study the performance of three diets (live marine zooplankton, *Artemia* spp., and commercial dry-powder feeds) were tested on the growth, feed utilization and survival rate of rabbitfish larvae and fry. The highest growth performance of rabbitfish larvae and fry in the early stages of rearing in terms of FBW, BWG and SGR was observed in the fish fed with live marine zooplankton, followed by fish fed with *Artemia* spp. (as shown in Fig. 6). It is possible that the mixed species of zooplankton provide a wider diversity and richness of nutrients suitable for growth of rabbitfish. Studies by Delbos (2019) and Giri *et al.* (2002) show that mixed species of zooplankton are richer in proteins (approximately 42-65 crude protein), carbohydrates, minerals and essential fatty acids to promote growth compared to a single species and therefore promote better growth. Further, protein quality and quantity determine growth and survival of larvae and fry of the reared fish. Also, the studies of Furuya *et al*. (2004) and Owodeinde and Ndimele (2011) reported that the difference in crude protein content between feeds or food causes the differences in growth performances of the fish in captivity. Besides the protein content, ideal culture conditions could also contribute to faster growth and high survival rate of larvae and fry.

In support of the above, a study by Yousif *et al*. (2005) and Yousif *et al.* (2014) reported that protein content and ideal culture conditions including good water quality parameters, promote faster growth of rabbitfish in captivity. In this study, the mixed species of zooplankton that were cultured in different manures provided good protein sources for fish larvae and fry. Previous studies by Refstie *et al.* (1998), Conceição *et al.* (2010) and Shaheen (2013) have shown that zooplankton are the preferred prey for fish larvae due to their good nutrition, flavour, texture, digestibility, palatability and attraction to predators. In this study, mixed live zooplankton such as daphnia, copepods, rotifers and *Artemia* spp. were present in culture experiments and were used in growth performance comparison.

The low growth performance, survival rate and slightly lower value of feed utilization efficiency of the fish fed on commercial feed could be due to the higher fiber content in feed used (7.3 %). A study by Ulloa and Verreth (2002) supported the fact that high dietary fiber levels could reduce growth performance, feed and protein utilization in fish and other domestic animals, regardless of amount of protein present. Also, low palatability and digestibility are considered possible causes for low growth performance, and this is more important for the first period of fish feeding, as reported by Ahmad *et al*. (2012). There was a slight difference between fish fed with live marine zooplankton and *Artemia* spp. Using mixed species of live zooplankton is crucial for fast growth, improved immunity to resist diseases and increased production due to higher survival rate of fish in the culture system. The intercepts of the graph in Fig. 6 has commercial implications in that dry feed can be used in the grow-out stage of rabbit fish culture. Commercial dry feeds promote healthy growth in juveniles, perhaps because zooplankton are very small to be fed by fish of this size. This was observed after 21 days in the present study where larvae and fry started to show a good response to commercial dry feed. This is similar to the results of the first study of this nature in the western Indian Ocean carried out by Bwathondi (1982) who reported that rabbitfish showed a good response when fed with artificial commercial dry feed, and grew better with these feeds during the juvenile and mature stages compared to other feeds.

In this study, the feed utilization efficiency recorded was within acceptable ranges for the growth of rabbitfish larvae and fry and other fishes such as Nile tilapia. Similar results were provided by Yousif *et al*. (2005) who reported an average value for FCR of 2.01 for rabbitfish fingerings fed with commercial dry ARASCO (Saudi Arabia) sinking feed. Also, studies by Ogunji and Wirth (2000) and Yousif *et al*. (2005) reported FCRs ranging from 1.19 to 3.5. Therefore, all FCRs obtained in this study (1.72-2.26) were within the acceptable ranges for the growth of rabbit fish larvae and fry.

Conclusion

The study revealed that there is good potential for culturing marine plankton (phytoplankton and zooplankton) by using media fertilized by manure and that the cultured marine plankton were a better source of food for rabbitfish larvae and fry than artificial feed during the onset of the feeding. It was observed that the types of manure used favour the growth of commercially important species of plankton such as the phytoplankton *Chaetoceros* sp., *Skeletonema* sp., *Chlorella* sp., *Isochrysis* sp., *Nannochloropsis* sp., and *Spirulina* sp., and the zooplankton *Eurytemora* sp., *Calanus* sp., *Oithona* sp., *Branchionus* sp., *Moina* sp. and ostracods that can be isolated, cultured as pure strains and used in mariculture. The study suggested that the use of cow dung provides excellent results and can be an alternative to chicken manure. However, other nutrients such as silica need be measured in the cow dung that are important for the growth of some genera such as diatoms. Phytoplankton culture can be used to ensure sustainable production of zooplankton in captivity for feeding larval stages of fish. For the growth and survival rate of rabbitfish *S. stellatus*, the study suggested the use of mixed species of zooplankton instead of single species in larvae and fry rearing, to improve growth and immunity of fish to resist diseases and further enhance the quality of fingerlings and development of mariculture hatcheries. The good response of the rabbit fish to commercial dry feed makes the species very suitable for culture in the western Indian Ocean region.

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