Original Article

Comparison of growth and survival rates of big blue octopus (*Octopus cyanea*, 1849) fed on natural and formulated diets in captivity

Nancy Iraba[1,2*], Saleh Yahya[1], Jerry Mang'ena[2,3], Fadhili Malesa[2,3]

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[1] Institute of Marine Sciences-University of Dar es Salaam, PO Box 668, Buyu, Zanzibar

[2] Aqua-Farms Organization, PO Box 22564, Dar es Salaam

[3] School of Aquatic Sciences and Fisheries Technology-University of Dar es Salaam, PO Box 60091, Dar-es-Salaam

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*** Corresponding author:**
iraba@afo.or.tz

Abstract
Comparative studies on growth and survival rates of *Octopus cyanea* fed on natural and formulated diets in captivity have never been conducted in Tanzania. This study aimed to investigate the growth and survival rates of *O. cyanea* using natural and formulated diets. The three formulated diets were made up of a mixture of sardines, fish waste, and alternating ratios of crab paste content across the different diet treatments. Treatment B had 75 % crab paste content, Treatment C had 50 % crab content and Treatment D had 0 % crab content while Treatment A was based on a natural diet of frozen crabs (*Scylla serrata*) and was used as a control. After five weeks of feeding, the effect of each diet was analyzed on growth performance and survival rates for the octopus. Results showed that there was a significant difference in growth rate in all the diet treatments ($H=13.243$, $p=0.004$, $DF=3$). Specific growth rates (SGR) were higher in octopuses fed in feed treatment A and lowest in feed treatment D. The survival rates were 100 % for treatment A and feed treatment B and 75 % for treatments C and D respectively. More research is needed to develop optimal nutritional diets for faster growth rates of *O. cyanea* in captivity.

Keywords: octopus, nutrition, formulated diets, captivity

Introduction
Octopus cyanea, also known as the big blue octopus, is one of the most common exploited species in the Western Indian Ocean regions (Guard and Mgaya, 2002; Roper and Hochberg, 1988a). *O. cyanea* is found within phylum Mollusca, class Cephalopoda, and family Octopodidae and is distributed throughout the Indo-Pacific Ocean from Hawaii to the eastern coast of Africa. Their preferred habitat is in either the lower reaches of the intertidal reef flat or along the reef edge where they live in small holes (dens) and crevices often hidden by small stones, rubble and pieces of shell (Norman, 2000; Guard and Mgaya, 2002). Octopus play an essential ecological role in the marine ecosystem where they act as predators and potential prey to larger fishes such as sharks and some neritic tuna (Forsythe and Hanlon, 1997; Guard, 2009). In Tanzania, octopus fishing is practiced by small-scale fishers and occurs extensively along the coast providing a source of protein and improving the livelihoods of the fishermen (Jiddawi and Ohman, 2002). Despite of the high economic value of the octopus fishery, global octopus catch declined to 335,865 tonnes in 2012 from 380,000 tonnes in 2007. In Zanzibar and Tanzania Mainland, octopus catch peaked at around 1700 tonnes in 2003 before dropping sharply to 703 tonnes in 2006 (FAO, 2014). The reported declines in octopus catches have been linked to several factors, including

over-exploitation for export markets, increasing number of octopus fishers and tourists, poor management practices, seasonal change in sea temperatures, habitat degradation, disease outbreaks, pollution and predation (Katsanevakis and Verriopoulos, 2006; Van Heukelem, 1973; Sparre, 1998; Rocliffe and Harris, 2016). In 2020, the global export of cephalopods amounted to USD 10.2 billion which is equivalent to 6.8 % of the total value of exports of aquatic products; this trend resulted in the share of cephalopods in global trade increasing over time and put supplies at risk due to poor management (FAO, 2022). Efforts have been focused on octopus fishery closures to assist in fishery sustainability, and little attention has been paid to exploring the viability of successfully culturing octopus species for commercial industrial production. The first feeding and behaviour experiments on the rearing of octopus in captivity were performed by the Instituto de Ciencias Marinas de Vigo and the Instituto Español de Oceanografía in Spain (Guerra, 1978; Nixon and Mangold, 1998). Octopus juveniles were grown in tanks and floating cages obtaining promising results and since then, many Spanish research centers have shown interest in the development of production techniques for octopus, such as Ciencias Marinas del Mar of CSIC, Barcelona (Villanueva, 1995). Such studies have not been explored across the Western Indian Ocean (WIO) region although octopus species present a series of favorable characteristics for rearing in captivity and commercial farming. These include: a great tolerance to captive conditions (Iglesias *et al*., 2000), very fast growth rate (Mangold, 1983; Van Heukelem, 1973; Guard, 2009, Roper and Hochberg, 1998b)

1988b), high feed conversion efficiencies (Mangold and Von Boletzky, 1973), high reproductive rate of up to 400,000 paralarvae (Boyle and Rodhouse, 2005) and a high market price (Vaz-Pires *et al*., 2004). Several cephalopod species have been studied worldwide, and various species of octopus have been reared in captivity to sexual maturity in the 1960s to 1970s, including *Octopus joubini*, *O. briareus* and *O. vulgaris*. In the early 2000s, the first successful commercial-scale octopus farming operation was established in the state of Yucatan for *O. maya* (Lopez *et al.,* 2015).

Nevertheless, making formulated feeds of appropriate nutritional composition is the main challenge to the development of aquaculture of octopus (Vaz-Pires *et al*., 2004; Cerezo and Garcia, 2016). A series of experiments have shown that formulated feed is the determinant of successful growth and intensification of octopus aquaculture production. So far, considerable effort has been made to develop artificial diets (Aguila *et al*., 2007; Domingues *et al.,* 2007; Cerezo *et al*., 2008). Future development of octopus aquaculture depends on the development of nutritionally sound and cost-effective feeds that can support production levels (Kirimi *et al*., 2016). Despite octopus being recognized as an ideal candidate for aquaculture, studies on octopus aquaculture in relation to formulated diets remain limited. This study aimed at investigating the performance of formulated diets on growth and survival rates of *O. cyanea* in comparison with a natural diet based on frozen crab. The selection of the ingredients to formulate experimental diets for the octopus was based on past successes reported in the literature from similar studies with different octopus species, and the availability of these ingredients locally in large quantities.

Material and methods

Description of the sampling site

The experiment was carried out for 35 days at the Institute of Marine Sciences, Buyu campus, Zanzibar. *O. cyanea* used in the study were obtained from local fishermen in Kizimkazi Mkunguni (Fig. 1) who used free diving to collect the specimens. Kizimkazi Mkunguni is located on the southern coast of Unguja Island, Zanzibar with geographical coordinates 6°26’60.0”S, 39°27’60.0”E and is characterized by sandy beaches and extensive nearshore coral reefs inhabited by abundant numbers of *O. cyanea*. Fishing is the main economic activity supporting livelihoods.

Experimental design and data collection techniques

Collection of juvenile Octopus cyanea

A total of 32 live juveniles of *O. cyanea* with an average weight of 20 to 250 grams were collected at a depth of 1 to 3 meters during low spring tide at Kizimkazi Mkunguni by free diving, the 32 octopuses were graded according to their weights and kept in 10L buckets separately in pairs. The buckets had openings in the lid to allow flow of air, while small pebbles were placed on the bottom to mimic the wild environment. No aerators were used during the approximately 2-hour transportation to the Institute of Marine Sciences, Buyu campus where the experiments took place. The octopuses were randomly distributed in sixteen tanks of 1000 L (two octopus per tank) in a flow-through saltwater system at the experimental site.

Water quality parameters (temperature, salinity, dissolved oxygen, and pH) were measured twice a day (morning and afternoon after feeding). Growth in weight was evaluated after every seven days. Coral fragments were kept inside the tanks to allow hiding and attachment as in the natural wild habitat. The octopuses were acclimatized for two days before the onset of the experiment and during this period they were not fed to allow them to get used to the captive conditions (after Sen, 2019).

Figure 1. Map of Unguja Island showing location of sampling area at Kizimkazi Mkunguni.

Experimental design

The experimental tanks were distributed randomly in the experimental site and labeled based on the feed treatment provided. There were three feed treatments; each with four replicates. The feeds used in this study were composed of sardines, fish waste, and varying amounts of crab paste in the different treatments. The crab paste content in Treatment B was 75 %, 50 % in Treatment C, and 0% in Treatment D. Treatment A used a natural diet of frozen crabs (*Scylla serrata*).

Each tank contained a PVC tube of 10.16 cm diameter that was placed at the bottom of the tanks for the octopus to use as a refuge. Other habitat substrates such as pieces of dead hard corals and pebbles were also kept in the tanks. Seawater was exchanged regularly to maintain a conducive condition for the survival of the *O. cyanea*. To prevent escape, each tank was covered with a plastic mesh lid designed with two removable hatches. Seawater inlet was through a pipe that was connected to the tank reservoir containing water from the sea. The seawater outlet was located at the end

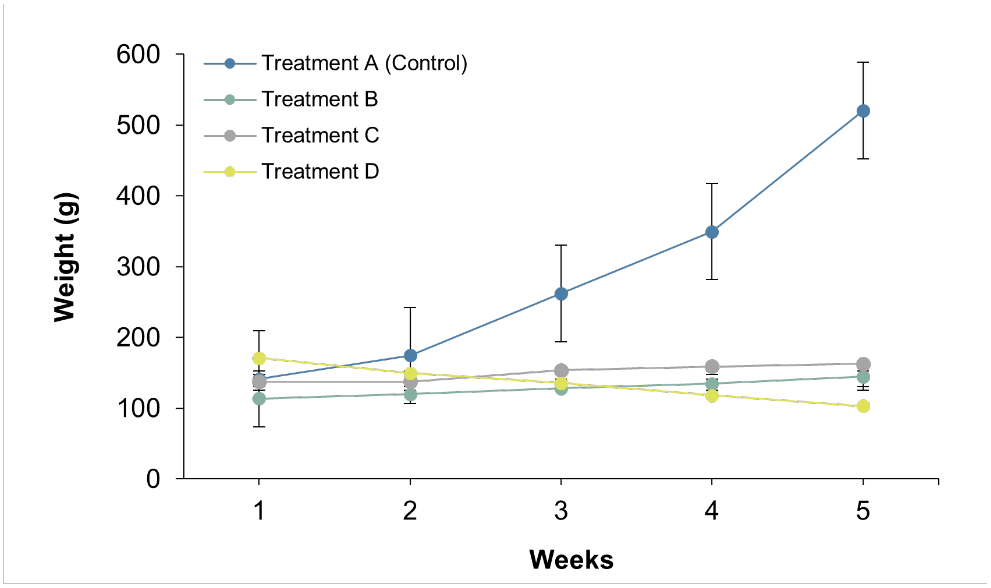

Figure 2. Mean weight of *Octopus cyanea* fed on formulated diets and frozen crab (Treatment A) for 5 weeks.

of each tank and sealed with a cork. The outlet was less than 2 cm diameter to prevent the octopus from escaping during opening and closing. Temperature, salinity, dissolved oxygen (DO), and pH were checked twice daily (morning and afternoon after feeding). The three formulated diet treatments and the control were supplied to the octopus once a day at (0900 hrs) at 5 % body weight for all regimens (after Farìas *et al.*, 2010). Unconsumed feed was siphoned every day (at 1700hrs). Overnight faeces was removed from the tanks before the octopus were given the initial feeding. The experiment was carried out for a period of 35 days.

Preparation of the formulated feeds for the experimental diets

Three formulated diets and a natural diet consisting of a crab diet (*Scylla Serrata*) were used in the experiment. The formulated diets (Fig. 3) were a mixture of sardines (*Sardinella longiceps*), fish waste that consisted of fish stomach contents, intestines, heads and fins that are often discarded at fish markets, and crab paste (soft parts of the crab meat after the skeleton has been removed). Cassava flour was used as a binder. Fifty kg of each ingredient was purchased, dried for 24 hours under the sun and ground using a grinding machine, then sieved using 0.5 mm mesh to remove indigestible parts and to obtain a fine powder for all ingredients. This was followed by mixing thoroughly by hand to form a uniform single mixture; however, for the case of crab, the hard parts including the carapace and hard shells which are less nutritious and less digestible were first removed before other processes. Formulated diets of different crab paste content (75 %, 50 % and 0 %) for the diet treatments were obtained. Hot boiled water was added to cassava flour as a binder

Table 1. Feed composition in % for *Octopus cyanea* formulated diets: crab paste, sardines and fish wastes.

Ingredients	Feed Treatment B	Feed Treatment C	Feed Treatment D
Crab paste	75.0	50.0	0.0
Sardines	12.5	25.0	50.0
Fish waste	12.5	25.0	50.0

Table 2. Proximate analysis for *Octopus cyanea* formulated diets: crab paste, sardines and fish waste.

Ingredient	Dry Matter	Ash	Crude protein	Crude Fiber	Ether Extraction
Crab paste	95.0±0.30	41.5±0.50	31.1±0.40	7.6±1.30	0.0±0.10
Sardines	94.4±4.90	38.3±0.50	47.2±0.20	0.6±0.30	4.9±0.20
Fish wastes	95.0±2.20	37.9±1.60	8.6±1.20	0.2±0.10	2.0±0.10

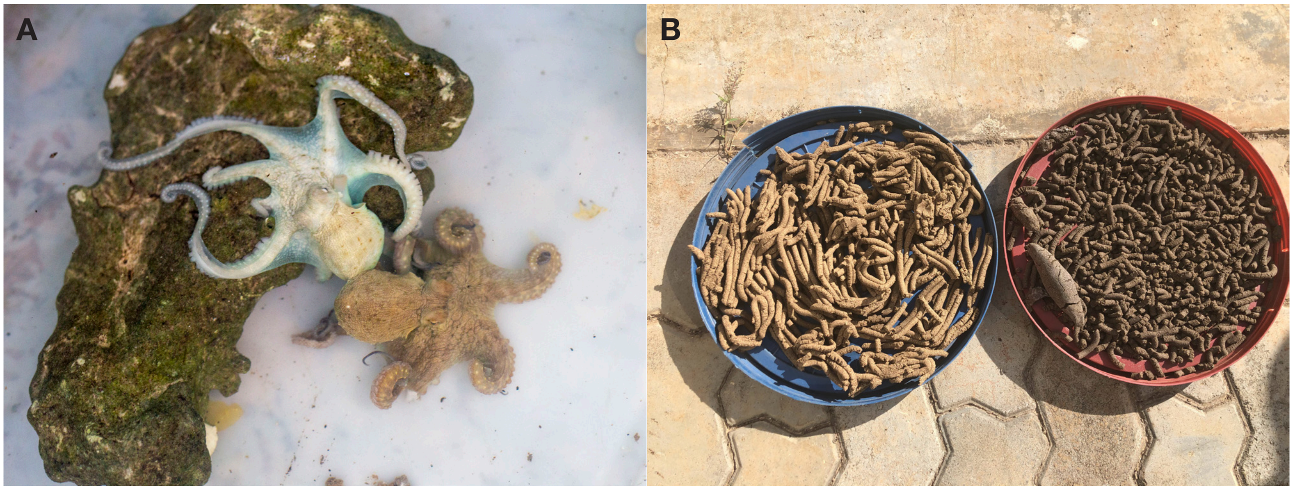

Figure 3. (A) Photo showing *O. cyaena* in a tank. (B) Photo showing formulated feed with crab paste content (left) and with no crab paste content (right).

to obtain separate doughs containing the various dietary ingredients. The dough was then passed through a pelleting machine to obtain 1.5mm diameter pellets. Immediately after pelleting the feeds, they were dried under the sun and then stored in an air-conditioned room at a temperature below 18 °C. The crabs were purchased from Darajani fish market in Zanzibar and then stored in the freezer at the experimental site at Buyu Campus. Proximate analysis for each feed ingredient was conducted at Sokoine University of Agriculture to assess their nutritional content (Table 2).

Data analysis

The data obtained are presented as mean ± SE (standard error). Calculations of growth performance parameters for the different feed treatments were conducted for Specific Growth Rates (SGR), Weight Gain (WG), Average Daily Gain (ADG) and Survival Rates (SR) by using the formulae below as described by Abarike *et al.* (2012). To determine the growth rates of *O. cyanea* fed on the different formulated diets, growth data was tested for normality and homogeneity by Shapiro's test of normality and Levene's test for homogeneity respectively, using R programme 4.6. The data were found not to be normally distributed therefore the non-parametric test of Kruskal Wallis was conducted followed by a post-hoc pairwise Wilcoxon test to depict where the significant difference in growth rates lies within the different diets.

Specific Growth Rate (% per day) = [(Ln final body weight – Ln initial body weight)] × 100/Experimental period.
ADG (g) = Weight gain/culturing days
Weight Gain (g) = Final weight (g) - Initial weight (g)
Survival Rate (%) = (Initial number of octopus stocked – mortality)/initial number of octopus stocked × 100

Results

Growth rates of *O. cyanea* fed on different formulated diets

Growth rates for *O. cyanea* were calculated and are presented in (Table 3) below, together with initial mean body weight (g), final mean body weight (g), weight gain (g), SGR (%/day) and ADG (g) with mean ± standard error. The highest SGR was displayed by

Table 3. The Growth performance of *Octopus cyanea* with different formulated feeds. Values show mean ± standard error.

Parameters	Feed Treatments			
	Feed Treatment A	Feed Treatment B	Feed Treatment C	Feed Treatment D
Initial mean body weight (g)	141.50±40.30	113.80±29.30	137.50±25.90	171.00±40.50
Final mean body weight	520.50±131.50	144.75±30.70	162.75±18.30	103.00±32.80
Weight gain (g)	379.00±106.80	25.00±7.70	31.25±17.30	-68.00 ± 15.20
SGR (%/day)	3.98±1.20	0.97±0.90	0.58±0.90	-1.58±0.50
ADG (g)	54.00±15.30	4.40±1.10	3.61±2.50	-9.70±2.20
Survival (%)	100	100	75	75

Table 4. Temperature, salinity, water pH, and DO of the experiment at different feed treatments.

Parameters	Feed Treatment A	Feed Treatment B	Feed Treatment C	Feed Treatment D
Temperature (°C)	25±0.20	26±0.20	24±0.20	26±0.00
DO (Mg/l)	9.0±0.10	7.0±0.20	8.5±0.60	7.4±0.00
pH	8.7±0.00	8.7±0.00	8.8±0.00	8.7±0.00
Salinity	38.9±0.20	38.5±0.00	39.0±0.20	40.0±0.10

a natural diet based on frozen crab followed by feed treatment B and C, with the lowest SGR displayed by feed treatment C (Fig. 1).

Survival rates

The survival rate was 100 % in treatment A and B, and 75 % in feed treatment C and D.

Water quality parameters

In all treatments the temperature ranged from 24 °C to 26.4 °C DO varied from 6.8 to 9.5 mg/l, pH varied from 8.6 to 8.8, and salinity from 38.5 to 40. (Table 4).

Discussion

Growth and survival rates of *O. cyanea* in captivity

Octopus showed a slow positive growth rate for feed treatments B and C while a negative growth rate was recorded in feed treatment D. There was no significant difference in growth rate shown in all the three formulated feeds (B, C and D). These results can be compared to a study by Martínez *et al*. (2014) in which best growth rates in juvenile stages of *O. maya* were obtained by using a moist crustacean-based diet and this was attributed to high protein assimilation and digestibility levels of this diet. Furthermore, Aguado and García (2002) reported that growth and food intake were higher with a crab diet when rearing *O. maya* in captivity. Other studies agree that the natural diet is still the most reliable, especially when based on crustaceans (Sánchez *et al*., 2014; Gutiérrez *et al*., 2015). The success of these studies are attributed to the longer period of the experiment. Replacement with formulated diets must be carried out using crustaceans-based diets, squid or fish, using freeze-dried meals (Estefanell *et al*., 2013; Rosas *et al*., 2013; Rodríguez *et al*., 2015). However, studies that have used a fish diet for octopus have exhibited lower growth rates and survival compared to those using a crustacean-based diet (Cagnetta and Sublimi, 2000; Domain, 2000; Aguado and García, 2002). The lowest growth rates have been attributed to low acceptability (López-Uriarte and Rios-Jara, 2009; Farías *et al*., 2010; Estefanall *et al*., 2013;), and low digestibility (Martínez *et al*., 2014). To date, species such as *O. maya* and *O. vulgaris* have shown acceptable developmental rates based on formulated moist feeds allowing them to replace fresh natural diets at the experimental level (Cerezo *et al*., 2008). This is evident after a series of feed experiments from the 1960s to date, exploring different nutritional requirements aiding fast growth. The present study provided initial experimental information on *O. cyanea* using diets that included locally formulated feeds in Tanzania. The results show a slow positive response and this provides room for more experiments with various feeds to determine the best nutritional requirements for octopus aquaculture. There was a significant difference in survival rates observed amongst feeds in this study; those fed with less crab had lower survival rates compared to those with more crab in their diet (100% survival).

In term of water quality, the salinity range for octopus is between 35 to 39.5 psu (Mangold, 1983). In this study, they survived well between 38 up to 40 psu. There were occurrences of temperature fluctuations recorded within the experiment, but they fell within *O. cyanea's* survival and growth range (24 - 26 °C). The present study did not estimate what temperature ranges supported the most efficient metabolic activities of the animals. For example, within the survival temperature range, *O. vulgaris* responded to temperature rises by increasing food intake and growth (Mangold, 1983); this can be an area of interest for future studies to compare different temperature ranges for *O. cyanea* growth and survival. The tanks were well aerated with aerators providing sufficient DO throughout the experiment, and there was regular water exchange twice a day, maintaining the DO at > 6 mg/l which proved to be within the survival range for *O. cyanea.* However, the species showed the ability to survive in low oxygen supply conditions for short periods. For instance, they endured 3 hours of transportation from the wild to the experimental site, with no aerators. Nevertheless, the release of ink into the water as a mechanism to defend themselves from predators (usually occurring during weighing operation) clouded the water, and the accumulation of this ink lowered the DO.

Formulated feeds and proximate analysis

O'Dor *et al*. (1984) reported a 96 % protein and 46 % lipid digestibility for *O. vulgaris,* indicating clearly that this species has the capacity to digest and assimilate protein rapidly. Crude protein (CP) is the major constituent and most costly component of fish feed (Kaushalendra *et al*., 2016). CP in feeds provides essential amino acids; it was the most significant nutritional composition amongst the three feeds in this study for promoting growth in the animals. The highest CP values were associated with sardines followed by crab paste while the lowest was found in fish waste. Studies by Mangold and Boletzky (1973), Aguado and Garcia (2002), and Villanueva *et al*. (2002) have shown that crustacean-based diets have high protein, assimilation and digestibility levels that favour growth and survival of octopus. Furthermore, lipid content is known as a limiting factor to growth, due to the low digestibility and assimilation levels of lipids by octopus. This suggests that for fast growth of octopus there should be appropriate matching of levels of crude protein and lipids content in the diet. In this study fish waste had the highest lipid levels (4.85 ±0.20), followed by sardines (1.99±0.06) and crab paste (0.04±0.13). Thus, despite the sardine having high crude protein content, crab paste has the right proportions of protein and lipid that favors positive growth of octopus. This is due to lower lipid content that enhances better digestibility and assimilation of the ingredients in octopus compared to sardines and fish waste. This is supported by Cerezo *et al.,* (2008) who reported that matching nutritional proportions of protein and lipids contributes greatly to high growth and survival rates of the octopus species.

Water quality parameters

Temperature, salinity and DO were observed to be the most important parameters to the growth and survival rates of *O. cyanea* in tanks. In this study the parameters were measured in the morning before feeding (0900hrs) and in the evening (1700hrs). The highest salinity recorded was 40 psu and the minimum was 38 psu. To maintain acceptable levels of DO water was exchanged in the morning before feeding and in the evening after feeding. Disturbances can cause octopus to release ink, thus clouding the water and lowering the DO (pers. obs.). The optimum range was observed to be within 6.68 - 9.05 mg/l; this was well maintained throughout the experiment through aeration. The pH was observed not to fluctuate as often as other water parameters, and throughout the experiment it remained between the safe range for *O. cyanea* of 8 - 8.5.

Conclusions

This study observed *O. cyanea* to have high selectivity and preference to the crustacean-based diets compared to the mixed non-crustacean diets (inclusion of fish waste and sardines). This selectivity and preference explains the prolonged time it took for octopus to adapt to the mixed ingredients, and explains the slow growth on the mixed diets with feed treatment B and C of 75 % and 50 % of the crab paste content respectively, and the negative growth for the feed treatment D of 0 % crab content. Treatment A showed fast growth because this diet contained the same content (crabs) as in the wild and did not require time for the octopus to adjust to it. However, the length of the experiment was only 5 weeks, a factor that may have affected the ability of octopus to adapt to the mixed ingredients, hence causing slow growth. Further research should consider the time factor of the experiments to determine appropriate feed combinations that work best for *O. cyanea* in captivity. Determination of the nutritional requirements for rearing *O. cyanea* is still at an infantile stage in Tanzania compared to other countries such as Spain. Therefore, this area remains largely unexplored and the suitable feed combinations that support highest growth levels are yet to be determined. Different countries have had trials with different feed mixtures in rearing different species of octopus which have provided good results over time, therefore future collaborations with countries that have pioneered successful trials should be established for more intensive research and experimental modifications that can contribute to octopus farming in the future.

Recommendations

The current study suggests the following recommendations in order to increase knowledge relevant to establishing octopus aquaculture with suitable feed formulation in Tanzania:

The current study assessed the effect of formulated feeds in *O. cyanea* in terms of growth and survival rates in captivity for only 35 days. Further studies should extend the culture period until the marketable size is attained to confirm any other effects associated with the formulated feeds.

Future research should focus on studying the matching nutritional requirements of *O. cyanea* for attaining higher growth rates in captivity; This aspect is still a challenge for octopus aquaculture research studies. Once the best feed combination with the correct nutritional quantities is determined octopus aquaculture can be successful. This requires resources in terms of funds, time and human capacity.

Other studies should focus on further research on replacing the "crustacean-based diet", which has proven crucial to the growth of *O.cyanea* and other octopus species worldwide, with an alternative, cheaper source which is nutritionally sufficient and acceptable to the animal. This is because crabs are expensive to purchase, and identifying an alternative best feed option will make this farming possible and affordable.

Different countries have had trials with different feed mixtures in rearing different species of octopus which have provided excellent results over time, therefore future collaborations with countries that have pioneered successful trials of octopus rearing should be established for more intensive research, knowledge exchange, resource mobilization and modifications.

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