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Full Length Research Paper

Growth performance and nutrient composition of *Bufo maculata* (Linneaus) tadpole fed different practical diets as fish meal substitute

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One thousand and one hundred (1,100) *Bufo maculata* tadpoles of weight range 0.03 -0.05 g (mean = 0.04 ± 0.008 g) and length range, 1.2 - 1.6 cm (mean = 1.4 ± 0.018 cm) were collected from the breeding tanks using scoop net and stocked into the outdoor concrete culture tanks coded according to the experimental diet at the rate of one hundred and twenty tadpoles of known weights and lengths per tanks and cultured for 84 days. The growth performances, feed utilization and nutrient composition of the tadpoles (*B. maculata*) fed three experimental diets namely live zooplankton alone (D1), 40% crude protein National Institute for Freshwater Fisheries Research (NIFFR) crumb diet alone (D2) and mixed diet (50% live zooplankton and 50% of dry 40% crude protein NIFFR crumb (D3) were studied. They were harvested after culture and processed into meal. The best mean weight gain (3.65 g/tadpole), specific growth rate (2.34%/day) and feed conversion ratio (2.05) were from tadpole fed mixed diets.

Key words: *Bufo maculata*, tadpole, growth, plankton, nutrient composition.

INTRODUCTION

The challenges for appropriate utilization of natural resources of either living or non- living from fauna or flora have rouse the interest of both national and international scientific agencies as this will go along way in sanitizing the natural environment or improve our technology. One of these challenges is the conversion of members of the family Bufonidae to useful consumeable product since man refusal of its consumption due to the reported toxins (Segun, 1989; Flier et al., 1980; Ely, 1994). These toxins which are concentrated in the 'warty' skin and two large glands one on each side of the toad's head, muscle, bones, body organs and eggs which have been documented to originate from the arthropod consumed by the toad/tadpole in their natural habitat. The toxins include 5,8-disubstituted indolizidines, a relatively common class of alkaloids from denbrobatid frogs, pyrrolidines, piperidines, izidines and 2,5-disubstituted decahydroquinolines (DHQs) from ants and spropyrrolizidines from millipede (Daly et al., 2002). These mean that some of the toad skin alkaloids may have been sequestered and retained from dietary source during the life cycle (Daly et al., 2002). To curb this there is a need for culture of tadpole in isolation.

Bufo maculata used for this study is one of the commonest toads found in the semi-arid zone of Nigeria (Segun, 1989). This animal has a very high reproductive rate and breeds during the rainy season alone in the wild. There is no competition for this resource by man in this part of the world. So, it can be used as fish feed ingredient. The possibility of replacing fish meal with toad and tadpole meal has been reported by Annune (1990) and Ayinla et al. (1994) but none of these authors investigated into either the culture or nutrient composition of this natural resource studied. About 3000 eggs which hatched under 48 h and developed into tadpole within few days was reported in one female toad (Miller, 2002). Tadpoles get oxygen via their gills. Most pond species graze upon rotting materials using their hundreds of teeth and filter microscopic particles out of the water with the use of their gills. They get most of their nutrition from decomposing organisms which are breaking down the material they ingest. They get little in the way of nutrients

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Table 1. Composition of the compounded diet fed to the tadpoles.

Ingredient	Composition (%)
Fish meal	27.95
Groundnut Cake	40.71
Maize	27.04
Palm oil	2.00
Bone meal	1.00
Vitamin Premix	1.00
Salt	0.30
Total	100.00

from the dead plant material that they are feeding upon. Organisms such as tadpoles that eat dead organic matter are called detritivores. Their massive population constituted nuisance to the environment most especially during rainy season while their voracious feeding habit and wide range of habitat adaptability are factors that aid the possibility of breeding toad in isolation (Obun et al., 2005). Utilizing the tadpoles for fish feed is a means of controlling the population.

The benefits derived from converting the tadpole into finished products in the provision of animal proteins at low cost will immensely contribute to the development of aquaculture in Nigeria by reducing the cost of feeding which has been report has about 40 - 60% of the total recurrent cost in aquaculture industry (Olomola, 1990; Falaye, 1992). This cost reported has been as a result of hike in prices of some of the fish feed ingredients especially clupeids which face human consumption competition, hence the need for this study.

MATERIALS AND METHODS

Collection of *B. maculata* and culture procedure

Three pairs of matured male and female toads each of weight range 100 - 150 g (mean = 122.5 ± 19.2 g) and length range of 9.5 – 10.8 cm (mean = 10.2 ± 1.1 cm) were caught at the bank of Kigera Reservoir of National Institute for Freshwater Fisheries Research (NIFFR) New- Bussa, Nigeria Hatchery Complex during their pairing activity at night using hand nets.

Stocking of breeding tanks

Three outdoor concrete tanks of dimension 1.0 m x 1.0 m x 0.75 m at the Hatchery complex of NIFFR were used for the breeding. The bottom of each tank was laid with sandy–loamy soil. The top of each tank was screened with wire gauze. Water from Kigera Reservoir which has passed through the recirculatory and biofiltration system in NIFFR Hatchery Complex was used to fill the concrete tanks to 0.3 m depth. Ten stands each of water hyacinth plant was introduced into the culture tank before stocking since from previous observation, toads have been noticed to use water hyacinth plant as breeding nest. Brooders of known weights and lengths collected from Kigera Reservoir were introduced into the breeding tanks at the rate of one pair (female and male) per tank immediately after capture for breeding. They were kept for 48 h for laying and hatching of eggs and were not fed after mating had commenced.

Stocking of culture tanks

Nine outdoor concrete tanks of dimension 1.0 m x 1.0 m x 0.75 m at the Hatchery complex of NIFFR were used for the culture. Seventy-two hours (72 h) after hatching of the eggs, one thousand and one hundred tadpoles of weight range 0.03 - 0.05 g (mean = 0.04 ± 0.008 g) and length range, 1.2 - 1.6 cm (mean = 1.4 ± 0.018 cm) were collected from the breeding tanks using scoop net and stocked into the outdoor concrete culture tanks coded according to the experimental diet at the rate of one hundred and twenty tadpoles of known weights and lengths per tanks.

Feeding and sampling of the tadpoles

Plankton collected with plankton net of 250 µm mesh size from the Plankton Reservoir of the Natural and Live Food Unit, Environmental Division of NIFFR was fed to all the newly hatched tadpoles twice a day for a week. After one week of acclimatization, three experimental diets namely: D1, live plankton alone; D2, 40% crude protein NIFFR crumb diet alone (see Table 1 for composition); and D3, mixed diet (50% live plankton and 50% of dry 40% crude protein NIFFR crumb) were fed to the tadpoles at 15% body weight (Lutz and Avery, 1999) twice a day (7.30 - 8.30 a.m and 6.00 - 7.00 p.m). The rations were adjusted weekly based on changes in body weight of the tadpoles for experimental period of 84 days.

Sampling of tadpole was done once in two weeks using hand net. Ten tadpoles were randomly selected for total weight and snout-vent length measurements. They were returned back into the tanks after measurement. Dead tadpoles were removed, counted, recorded and discarded.

Monitoring of water quality used for tadpole culture

Water temperature was taken daily before feeding between 7.00 - 8.00 a.m. with graduated Mercury-in-glass Thermometer. Turbidity was determined weekly using Hach turbidometer in NTU (Nephelometric turbidity unit). The pH of the samples was determined weekly using a standardized pH meter (Behrotest model E251) while dissolved oxygen was determined using the Winkler Solutions as described by Boyd (1990).

Harvesting of tadpoles

The water in tanks was drained and the tadpoles were scooped out of the tanks with hand nets. Total cropping of the tadpoles was done. The total weight of the cropped tadpoles was taken while ten tadpoles were randomly sampled for snout-vent length measurement.

Processing and determination of proximate composition

The harvested tadpoles were weighed fresh and oven dried at 80°C for 6 h following the method of Akpodiete and Okagbare (1999). They were reweighed, milled into powdered form, packed in three airtight plastic bowls and stored in a refrigerator till when needed. The processed tadpole meal and fish meal (clupeid) were analysed for crude protein, crude fibre, crude lipid, ash, nitrogen free extracts and gross energy according to Association

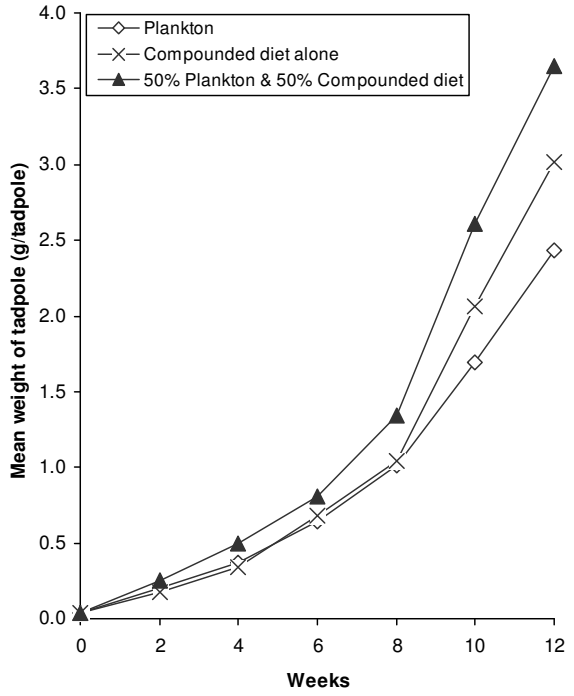

Figure 1. Bi-monthly growth pattern of *Bufo maculate* tadpole cultured in outdoor concrete tank.

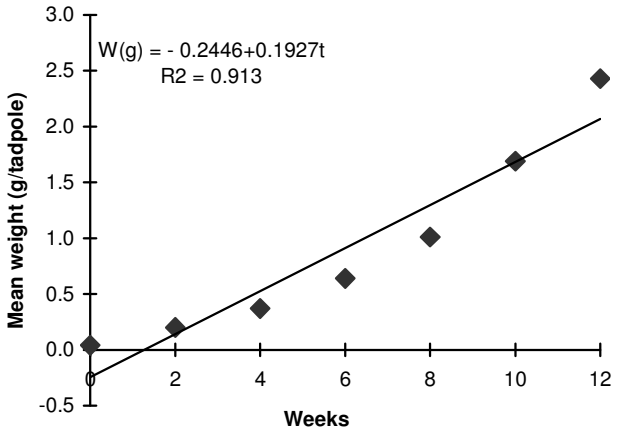

Figure 2. Linear regression of bi-monthly growth of tadpole fed Plankton alone.

of Official Analytical Chemist Methods (A.O.A.C., 2000). The minerals in the ash of each diet was brought into solution by wet digestion using Conc. HNO_3 (63%), perchloric acid (60%) and sulphuric acid (98%) in the ratio of 4:1:1 (Harris, 1974). Potassium and sodium was determined using flame photometer (Allen, 1974). Phosphorus was determined using Spectronic 20E, while magnesium by Perkin Elmer Atomic Absorption Spectrophotometer Model 2900.

Calculation and statistical analysis

At the end of the culture period, the growth rates, condition factor, survival rate and nutrient utilization of tadpole were computed according Fasakin et al. (2000), Hepher (1988), Becker et al. (1999), Burel et al. (2000) and Bagenal and Tesch (1978).

Weight gain/fish (g/fish) = $W_f - W_i$

W_f and W_i = final and initial weight at the end of and beginning of the experiment respectively.

Relative growth rate (%) = $[(W_f - W_i)/ W_i] \times 100$

Specific growth rate (%) = $[(\text{Log } w_f - \text{Log } w_i)/t] \times 100$

Where log w_f and log w_I are logarithm of final weight and initial weight respectively and t is experimental period.

Condition factor (k) = $(100W) / l^3$

Where W is the weight and l is the length.

Survival rate {S.R (%)} = $[(N_f) / N_i] \times 100$

Ni = Number Stocked at the beginning of the experiment, N_f = Number alive at the end of the experiment.

Feed conversion ratio (FCR) = Feed intake (g) / Weight gain (g)

All data collected were subjected to analysis of variance (ANOVA). Comparisons among treatment means were carried out by one-way analysis of variance followed by Kramer-Tukey multiple comparison tests. Standard deviation (SD) was calculated to identify the range of means. Least Significance differences (LSD) was used to determine the level of significance among treatments. Correlation and regression analysis was carried out to determine the relationship between the treatments and some of the parameters using SPSS 10.0 Windows 2000 and Graph pad Instat packages.

RESULTS

The mean values of water parameters recorded in the outdoor culture tanks of tadpoles were temperature range from 23.05 - 23.90°C (mean = 23.5 ± 0.75°C), dissolved oxygen range from 2.18 - 2.28 mg/l (mean = 2.25 ± 0.03 mg/l), turbidity range from 0.13 - 0.17 NTU (mean = 0.15 ± 0.01 NTU) were recorded during the experimental period. The weight of tadpole increased gradually from the beginning of the experiment to the 8th week and then sharply to the end of the experiment (12th week) in all the trials as shown in Figure 1. There were high correlations r = 0.956, 0.936 and 0.942 ($p < 0.05$) between weekly weight of tadpole fed Plankton alone, compounded feed alone and 50% plankton and 50% compounded feed diet, respectively, and experimental period. The linear regressions of weight with time are illustrated in Figures 2, 3 and 4.

The highest mean weight gain of 3.61 g/tadpole was recorded in 50% plankton and 50% compounded feed diet fed tadpole while the lowest (2.39 g/tadpole) was from tadpole fed plankton alone diet (Table 2). Highest and lowest relative growth rate 902.5% and 597.5% were recorded from tadpole fed 50% plankton and 50% compounded feed and plankton alone diets, respectively. There was significant difference ($p < 0.05$) between the

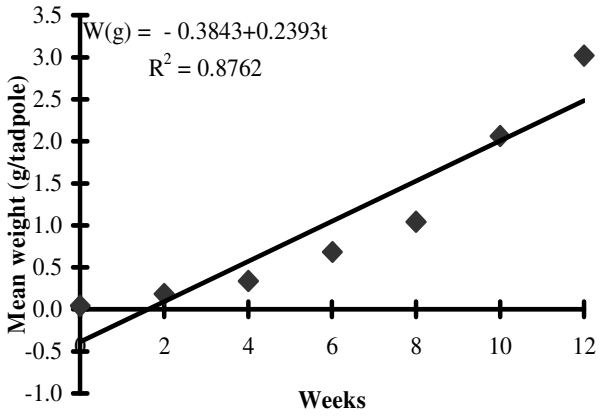

Figure 3. Linear regression of bi-monthly growth of tadpole fed compounded feed alone.

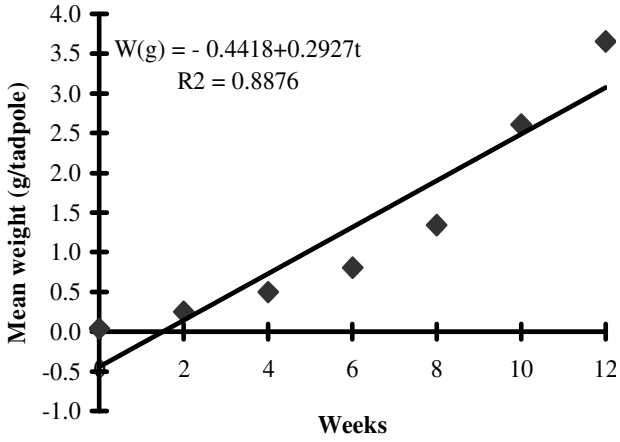

Figure 4. Linear regression of bi-monthly growth of tadpole fed 50% plankton and 50% compounded feed diet.

mean weight gain and relative growth rate by the tadpoles fed different diets. The specific growth rate ranged within 2.34 - 2.12%/day. There was significant difference ($p < 0.05$) between specific growth rate recorded in 50% plankton and 50% compounded feed and plankton alone diets.

Highest and lowest final condition factors, 1.78 and 1.25 were from tadpoles fed 50% plankton and 50% compounded feed and plankton alone diet, respectively. There was significant difference ($p < 0.05$) between the highest and lowest final condition factors. The survival rate ranged within 75 - 88.33%. There was significant difference ($p < 0.05$) between the survival rate recorded 50% plankton and 50% compounded feed and plankton alone diet respectively during the experiment.

The feed conversion ratio ranged from 2.05 – 2.47 and significantly differed ($p < 0.05$). The highest and lowest gross efficiency of feed conversion, 48.78 and 40.49% were recorded from 50% plankton and 50% compounded feed and plankton alone diet, respectively. There was significant difference ($p < 0.05$) between the gross efficiency of feed conversion recorded during the experiment.

Fishmeal was higher in crude protein and gross energy content 71.64% and 2074.73 kJ/100 g than tadpole meal 43.50% and 1639.63 kJ/100 g. Tadpole meal was higher in crude lipid, fibre, ash and nitrogen free extract than fishmeal as shown on Table 3. There was significant difference ($p < 0.05$) between nutrient composition of fishmeal and tadpole meal.

DISCUSSION

The fact that growth was reported from each of the dietary treatment indicate that tadpoles like fish can also be raise in isolation using concrete tank as against their natural earthen aquatic environment using any of the dietary treatments. Lutz and Avery (1999) had made similar report on the possibility of culturing toad and frog in isolation while Benitez-Mandujano and Flores-Nava (1997) and McCallum and Trauth (2002) reports showed the possibility of raising tadpole of *Rana catesbeiana* (shaw) and *Rana sylvatica* respectively on different diets. The highest mean weight gain, 3.61 g/tadpole reported in this study is lower though not significantly different ($p > 0.05$) from the mean weight gain of 3.65 g/tadpole documented by Benitez-Mandryano and Flores-Nava (1997) despite the fact that this weight was reported in tadpole given mixed diet comprising of phytoplankton and 13% body weight supplementary diet. The result from this study agreed with Brown and Rosati (1997), Le Than Hung et al. (1999), Ugwumba et al. (2001), McCallum and Trauth (2002) that aquatic animals (tadpoles of toad, frog and fish) grow better when fed mixed diet containing live food and compounded diet than when fed either live feed or compounded feed alone. This is not far from the fact that the synergetic effect of combined biological compounds is higher and superior than when that of individual compound. This observation is in agreement with suggestions by previous authors that combined protein source is better than single protein source for fish diets (Sigh et al., 1978; Mazid et al.,1987; Hossain and Juancy, 1989). Earlier than these reports, Reichenbach-Klinke (1972) observed that live organisms contribute significantly in the digestion of food by supplying digestive enzymes and also increase the conversion efficiency of compounded feed when fed as combined diet hence the best growth performance, feed conversion ratio and condition factor. The tadpoles fed compounded feed would have been expected to show the best growth performance since its contains fishmeal which has high level of protein that has been known as the best feed for animals (Lovell, 1994; Massomotu, et al., 1996), but this was not so. However, Lovell (1994) reported that the biological value of protein source does not only depend on its amino acid profile but also on its digestibility, which is a factor of nutrient utilization. Similar observations were made by Benitez-Mandujano and Flores-Nava (1997) and

Table 2. Growth performances and feed utilization of tadpole fed different experimental diets in outdoor tanks.

Parameter	Plankton alone	Compounded feed alone	50% Plankton and 50% compounded feed
Total initial weight (g)	4.8	4.75	4.8
Mean initial weight (g/tadpole)(SEM)	0.04	0.04	0.04
Total final weight (g/tank)	257.58^{b}	271.80^{b}	350.40^{a}
Mean final weight (g/tadpole)	2.43^{b}	3.02^{ab}	3.65^{a}
Total weight gain (g/tank)	252.78^{b}	267.05^{b}	345.60^{a}
Mean weight gain (g/tadpole)	2.39^{c}	2.98^{b}	3.61^{a}
Mean Initial length (cm)	1.4	1.4	1.4
Mean final length (cm)	5.8	6.1	5.9
Mean increase in length (cm)	4.4	4.7	4.5
Relative growth rate (%)	597.5^{c}	745.0^{b}	902.5^{a}
Specific growth rate (%/days)	2.12^{b}	2.24^{a}	2.34^{a}
Initial condition factor (k_1)	1.46	1.46	1.46
Final condition factor (k_2)	1.25^{a}	1.33^{a}	1.78^{b}
Feed conversion ratio	2.26^{ab}	2.47^{b}	2.05^{a}
Gross feed conversion efficiency (%)	44.25^{ab}	40.49^{b}	48.78^{a}
Number stocked	120	120	120
Number harvested at the end of the experiment	106^{a}	90.0^{b}	96.0^{ab}
Survival rate (%)	88.33^{a}	75.0^{b}	80.0^{ab}
Experimental period	84	84	84

All data on the same row with different superscripts are significantly difference ($p < 0.05$).
Data without superscript are not significantly different ($p > 0.05$).

Table 3. Proximate and mineral composition (% dry weight) of tadpole and fish meal (clupeid).

Composition	Unskinned-raw tadpole meal	Fish meal (Clupeid)
Crude protein (%)	43.50^{e}	71.46^{a}
Crude lipid (%)	11.30^{b}	7.97^{c}
Crude fibre (%)	3.8^{c}	1.18^{d}
Ash (%)	26.45^{a}	7.33^{b}
Nitrogen free extract (%)	8.15^{c}	3.17^{e}
Moisture (%)	6.80^{b}	8.89^{a}
Dry matter (%)	93.20	90.21
Gross energy (kJ/100 g)	1639.63^{d}	2074.73^{b}
Calculated gross energy (protein) (kJ/100 g)	37.69^{b}	29.03^{c}
Metabolizable energy (kJ/100 g)	1229.72^{c}	1556.05^{b}
Digestible energy (kJ/100 g)	1373.4^{d}	1812.7^{a}
Sodium (g/100 g)	0.61^{c}	0.91^{b}
Calcium (g/100 g)	2.51^{b}	3.53^{a}
Potassium (g/100 g)	0.21^{d}	0.96^{b}
Phosphorus (g/100 g)	0.57^{c}	2.4^{a}
Magnesium (g/100 g)	0.58^{a}	0.08^{c}

All values on the same row with the different superscripts are significantly different ($P < 0.05$).

McCallum and Trauth (2002) in their studies.

The low mortality coupled with better condition factors recorded for each dietary treatment indicated that each of the dietary treatment is accepted by the tadpoles and had no adverse effects on them. This was also observed by Faturoti (1991), Ugwumba et al. (2001) and Sogbesan et al. (2005). The best survival recorded in the plankton alone fed tadpole is in agreement with the report of Durary and Bagarino (1984) that live food enhanced survival of at the early stage of fauna.

The protein content in tadpole is high enough to serve as single animal protein source needed by *Heterobranchus longifilis* for proper growth and development which is the basic nutrient that cannot be compromised in the choice of ingredients for feed formulation and preparation (Zeitler et al., 1984). Protein has also been the reported as the most costly nutrient in fish diet. The nutrient quality of feed ingredient is one of the major prerequisite apart from availability before such ingredient is recommended for feed production. The crude protein content recorded in tadpole could compete well with that of other alternative protein supplements of animal origin fed to fish (Wee, 1988; Fasakin et al., 2000; Ugwumba et al., 2001; Madu and Ufodike, 2003; Sogbesan et al., 2005; Sogbesan et al., 2006) which indicates that feeding it to fish will not pose the problem of malnutrition. The result of this study also showed that tadpole is rich in lipid and feeding fish with high lipid diet may expose the fish to risk of fat deposition in the organs which impairs fish health as reported by Desilva and Anderson (1995) and Tacon (1987). The higher crude fibre in tadpole than fish meal may be one of the limiting factors to its utilization as a whole meal due to the fact that large amount of crude fibre in fish diet may dilute the feed nutrients, impair digestibility and decrease pellet quality (Dupree and Hunner, 1984).

Economic and nutritional importance of clupeids had lead to its over fishing, causing depletion in the yield from the wild which prompted government to enact a policy that placed embargo on its fishing. This had now led to increase in the cost of animal protein in fish diets. With the result so far from this study, tadpole which could be cultured in isolation with reduced cost and having nutrient value that appears to be less than that of fish meal but can still supplied the required crude protein of 42.5% by catfish and 30 -35% by cichlids. Hence the culture and the utilization of tadpole meal as fishmeal substitute is hereby recommended to interested fish farmers, feed millers and other fisheries stake holders.

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