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Growth and survival of Ripon barbel (*Barbus altianalis*) larvae and juveniles fed five experimental diets in captivity

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ABSTRACT

Mass production of quality seed is vital for commercial culture and requires prior knowledge of appropriate larval diets and their utilization. Four experiments were sequentially conducted at different periods to evaluate the effect of live and a processed microdiet on growth and survival of *Barbus altianalis* larvae and juveniles. Larvae were fed exclusively on live prey (*Moina* and *Artemia nauplii*), microdiet (57 % Crude Protein), decapsulated *Artemia* cysts and in combination (*Moina* + *microdiet*). The effect on growth was further evaluated in subsequent juvenile trial by co-feeding. Green water effect on larval growth was also evaluated. In the final experiment, 15 day old larvae were raised in fertilized outdoor concrete tanks. Results indicated that each diet affected larval growth significantly different ($P < 0.05$) with the combination diet (152.05 ± 2.51mg) and decapsulated *Artemia* (141.14 ± 2.43 mg) performing better than microdiet, *Moina* and *Artemia* nauplii in that order. In subsequent juvenile experiment, larvae originally fed decapsulated *Artemia* (510.13 ± 11.93 mg) and those fed a mixed diet (500.20 ± 11.8 mg) performed better than other diets. Ontogenetic pattern of amylase, lipase and protease activity identified larvae maturation age at 14–21 Days after hatching (DAH) (14.93 ± 0.36–31.5 ± 0.61 mg) with the combination diet. When larvae at 15 DAH were nursed in outdoor tanks, final survival and growth performance increased to 95.3 % and 1112 ± 42.70 mg compared to the indoor nursing at 90.9 % and 355.33 ± 6.44 mg respectively by 75 DAH. Therefore we recommend that any microdiet manipulations and or outdoor nursing be done during or after this period. Microalgae had no direct effect on larval growth ($P > 0.05$). In this study, larvae were confirmed to utilize the microdiet from exogenous stage but co-feeding produced best average weight (152.05 ± 2.51mg), specific growth rates (4.06 ± 0.19) and survival (90.9 %). This study provided guiding strategies for improved rearing of *B. altianalis* fingerlings in captivity.

1. Introduction

The Ripon barbell *Barbus altianalis* also locally known as Kisiinja is a high value native carp to Ugandan water bodies, majorly the lakes Victoria, Edward and associated rivers and streams (Aruho, 2018). The fish is cherished for its aroma when fresh or smoked and is a delicacy largely in the south west, central and eastern regions. It has successfully been domesticated but its commercial production is limited by availability of sufficient quality seed from hatcheries.

To promote hatchery rearing of a particular species it is imperative to identify effective diets provided at appropriate period for better development and growth of larval fish (Cahu and Zambonino-Infante, 2001; Herath and Atapaththu, 2013; Bisht et al., 2013). The sustainable production of profitable quality seed also entails reducing production costs by gradually orientating larvae to cheaper diets while maximizing their growth and survival (Mokolensang et al., 2003; Herath and Atapaththu, 2013). This is dependent on the availability of suitable diets that are readily consumed and efficiently digested to support rapid growth (Giri et al., 2002; Mokolensang et al., 2003). Large quantities of larvae cannot be sustained on live cultures alone as they are expensive to prepare and

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require considerable time to maintain (Kolkovski, 2001; Conceicao et al., 2010; Liu et al., 2012). They may also, at times, carry harmful disease agents (Lahnsteiner et al., 2009). Processed microdiets (dry feeds) are preferred because they are cheaper and easier to apply for weaning larvae (Chatain, 1997).

A good larval weaning strategy would enable the introduction of microdiets at a particular time and stage when fish can easily digest and utilize the feeds efficiently. This process varies among species as the gradual acceptance of food and digestive capability depend on developmental progression of the gastrointestinal tract and its capacity to digest and absorb nutrients (Cahu and Zambonino-Infante, 2001; Kujawa et al., 2010). In cyprinids such as *Leociscus* species (Wolnicki, 2005; Kwiatkowski et al., 2008), *Scardinius erythrophthalmus* (Wolnicki et al., 2009), *Aspius aspius* and *Chondrostoma nasus* (Kujawa et al., 2010), *Rutilus frisii kutum* (Falahatkar et al., 2011) and *Cyprinus carpio* (Mahfuj et al., 2012), larval growth and survival rates were strongly and differently affected by both the type of diet given and the age when food was introduced. An improper weaning diet could impair or delay the development of the gastrointestinal tract causing chronic stress leading to physiological malfunctions or even death (Cahu and Zambonino-Infante, 1994).

Cost-effective weaning strategies are focused on partial or total replacement of live diets by micro particulate diets (Liu et al., 2012). While some species can successfully be started on processed microdiets exclusively others are weaned on a combination of live diets and microdiets (Kolkovski, 2001). Co-feeding of live prey and microdiets may enhance enzyme activity to aid digestion of the microdiet and facilitate maximum uptake of nutrients since some of the larvae lack sufficient endogenous enzymes (Dabrowski, 1984). It is imperative to assess species larvae nutritional requirements and their comparative response to various diets, to ascertain and identify appropriate weaning strategies. Most of the commonly cultured live preys for fish larvae include rotifers, *Artemia* nauplii, copepods and cladocerans that are maintained on algal cultures (green water) for their nutrient enrichment (Das et al., 2012). However, some studies report a direct role of algae in growth and survival as part of the larvae weaning strategy (Reitan et al., 1997; Sanaye et al., 2014). The *Artemia* is said to be a good diet that can produce quality larvae for some cultured fish species but the high costs and importation encumbrances are prohibitive for most farmers in developing nations. Alternative, cheaper options could be a better strategy (Olurin and Oluwo, 2010).

In spite of its successful breeding using induced spawning techniques, *B. altianalis* exhibited slow growth and low larval survival (Rutaisire et al., 2015; personal observation) limiting its mass production. Low survival rates may be attributed, in part, to lack of knowledge on larval rearing. Recent investigations by Aruho et al. (2019) on ontogenetic development of the digestive system using histology revealed that *B. altianalis* larvae may have the capacity to digest a microdiet by 7 days post-hatch. However, effective utilization of diets to improve growth and survival of larvae required further experimentation. The performance of weaning diets can be evaluated through growth and survival parameters and also by determining ontogenetic patterns in enzyme activity to identify the earliest age of digestive competence of larvae provided for a given diet (Zambonino-Infante et al., 2008). In this study experiments were conducted to evaluate survival and growth of *B. altianalis* larvae raised on live prey, a microdiet, and decapsulated *Artemia* cysts. The study used enzyme assay techniques to evaluate changes in amylase, protease and lipase activity during larval development to establish the earliest period the digestive system matures and when it can utilize different diets. Based on the growth performance and digestive maturation competence level of larvae, this study determined the best growth rates, survival and appropriate period for stocking or nursing larvae in outdoor rearing facilities. The findings were of great significance in developing a weaning strategy for production of *B. altianalis* fingerlings under culture conditions.

2. Materials and methods

2.1. Experimental study 1; Larvae and Juvenile growth of Barbus altianalis

This experiment was conducted in two consecutive phases using the same subjects. In Experimental phase one (Experimental 1-Phase I), 6 day old larvae (6 DAH) were fed different diets to evaluate the diet effects on their growth and survival. In Experimental 1(Phase II), when the larvae were 48 DAH (Juvenile stage) they were all gradually introduced to the same diet (a combination of live and dry) to determine consequential effects or performance of larvae diets provided at larvae stage on growth of Juveniles.

2.1.1. Experimental study 1 (Phase I): growth and survival of larvae on weaning diets and diet combinations

The *B. altianalis* hatchlings for the experiment were obtained by inducing one ripe female using a running water technique described by Rutaisire et al. (2015). Eggs were fertilized and incubated at 27°C in a basin until hatch after 77degree days (67 h). The larvae were kept in the hatching basins at 27 ± 1°C and the ammonia level (monitored by LaMotte Fresh water aquaculture kit Code 6665-02-CC) was kept below 0.1 ppm by flow through of water until the yolk was gradually reabsorbed at 6 days after hatch (DAH). Fifty-five larvae (3.0 ± 1.0 mg) were randomly allocated to 15 glass aquaria of 55 l capacity each. Five diet treatments, each in triplicate, were randomly assigned to the 15 glass tanks. The treatments were as follows; *Moina* alone (MO), a combination of *Moina* + microdiet (MD), hatched *Artemia* nauplii (HA), decapsulated *Artemia* cysts (DA), and microdiet or dry feed (DF) alone. Diet DF was a commercial feed of 57 % crude protein (CP). Nutritional composition of the DF, including fatty acid and the amino acid profiles was analyzed at the nutritional laboratory of the University of Ghent, Belgium and Nitrilab Netherlands respectively (Table 1). Temperature in all experimental tanks was maintained at 27 ± 1°C using thermostatic heating rods (Sera Aquarium heater thermostat; sera D 52518, Heinsberg Germany). Ammonia levels were maintained below 0.1 ppm by cleaning tanks and exchanging water twice daily at 7:00 h and 17.00 h. Dissolved oxygen (DO) was maintained at 7.0 ± 2.7 mg/l throughout the experimental period. The hatched larvae were fed to satiation, 3 times a day at 8.00 h, 12.00 h and 18.00 h (Table 2). For the combination diet, larvae were fed *Moina* + microdiet (MD) at the same time during each feeding time. The experiment was conducted for a period of 48 days (48 DAH). About 45 larvae were sub sampled from each tank throughout the experiment at every sampling. The wet weight of each larva was recorded to the nearest 0.001 g using an electronic micro weighting scale (Model DJ V320A; 320 g/0.001 g, PAN SCALE Hardware) and the length was recorded to the nearest 0.1 cm using a calibrated ruler.

2.1.2. Experimental study 1(Phase II): consequential effects of larvae diets in growth of juveniles

This experiment was a continuation of the first experiment (experimental study 1-Phase I) whereby after terminating feeding the larvae with diets MO, MD, HA, DA, and DF; a co-feeding diet MD that performed well in Phase I was introduced to all the larvae in all the treatments (experimental tanks). The co-feeding diet MD was introduced at a stage when the larvae guts started coiling (Aruho et al., 2019). This is a period when larvae mortalities were drastically reduced to less than 20 % (see results section) in all the treatments, and when scalation process had began in larvae. This was presumed to be the beginning of juvenile stage. The experiment determined the effect (or the influence) on growth performance of juveniles (consequential effects) as a result of differences in diets MO, MD, HA, DA, and DF provided at larvae stage in the experiment 1 (Phase I). The experiment was conducted from 48 DAH to the 92 DAH in the same indoor glass tanks (aquaria). At 48 DAH, the diet MD was gradually used to replace the original diets in experiment 1-Phase I (Table 2) for 5 days until when the original feeds were

Table 1
Fatty acid and amino acid profiles of microdiet (dry feed) DF.

FAME: procentual composition & quantitation (mg/g dry weight)						Protein profiles	
Fatty acids	Area%	mg/g DW	Fatty acid	Area%	mg/g DW	Amino Acids	%/100g
14:0	6.4	9.4	I.S.			Alanine	3.32
14:1(n-5)	0.2	0.3	21:0			Arginine	3.12
15:0	0.6	0.9	20:3(n-6)	0.1	0.1	Aspartic acid	4.91
15:1(n-5)	0.2	0.2	20:4(n-6)	1.1	1.6	Cystine	0.65
16:0	18.9	27.6	20:3(n-3)	0.1	0.1	Glutamic acid	9.85
16:1(n-7)	7.0	10.2	20:4(n-3)	0.7	1.0	Glycine	3.47
17:0	0.7	1.1	22:0	0.1	0.1	Histidine	1.18
17:1(n-7)	0.1	0.2	20:5(n-3)	15.5	22.6	Isoleucine	2.28
18:0	4.2	6.2	22:1(n-9)	0.5	0.8	Leucine	4.21
18:1(n-9)	9.5	13.9	22:1(n-7)	0.2	0.3	Lysine	3.93
18:1(n-7)	3.1	4.5	23:0			Methionine	1.51
18:2(n-6)-t	0.1	0.1	21:5(n-3)	0.6	0.9	Phenylalanine	2.3
18:2(n-6)-c	3.6	5.3	23:1(n-9)			Proline	3.26
19:0	0.2	0.2	22:4(n-6)			Serine	2.57
18:3(n-6)			22:3(n-3)			Threonine	2.36
19:1(n-9)	0.2	0.3	22:5(n-6)	0.3	0.4	Tyrosine	1.59
18:3(n-3)	0.8	1.2	22:4(n-3)			Valine	2.38
18:4(n-3)	2.2	3.3	24:0			Tryptophan	0.58
20:0			22:5(n-3)	2.1	3.1		
20:1(n-9)	0.8	1.2	24:1(n-9)				
20:1(n-7)	0.3	0.4	22:6(n-3)	11.5	16.8	Proximate analysis	
			Sum (n-3) >or = 20:3(n-3)	30.4	44.5	Crude Protein	57 %
			Sum (n-6) >or = 18:2(n-6)-t	5.1	7.4	Crude Fiber	11 %
						Crude fat	15 %
			g wet	0.0579	0.0531	Ash	11 %
			% DW	91.645	9.4196	Phosphorous	18 %
			Total mg FAME/g DW		146.2	$CuSO_4$	8 mg/kg
			Total lipid % on DW			VitaminA ui/kg	70,000

Table 2
Feeding schedule (estimates) for different diets at satiation during experimental period.

Experiment 1a; (live prey estimates per individual)							
Days of culture	Week	*Moina* (MO)	*Artemia* nauplii (HA)	Microdiet) DF (g)	*Moina* + dry feed (MD) *Moina*	Dry feed (g) per tank	Decaps. *Artemia*
5–7	1	30–60	115–400	0.4	20–30	0.2	20–30
8–14	1	136–300	115–400	0.8	70–100	0.4	20–30
15–21	2	200–400	200–600	0.9	100–200	0.5	30–60
22–29	3	300–500	400–600	1	150–250	0.6	40–80
30–37	4	500–600	500–800	1	250–400	0.6	70–90
38–45	5	700–1000	600–1200	1	400–600	0.6	90–100
46–48	6	1000–1500	>1200	1	600–700	0.7	>100

Experiment 2; (*Moina* +Microdiet) proportions provided during transition period					
	Treatments				
Days of culture-transition	MO+Microdiet	HA+*Moina* &Microdiet(50%+50%)	DF +*Moina*	MD+*Moina* & microdiet (50 %+50 %)	DA+*Moina* & microdiet (50 %+50 %)
48	+20 %	+20 %	+20 %	100 %	+20 %
49	+40 %	+40 %	+40 %	100 %	+40 %
50	+60 %	+60 %	+60 %	100 %	+60 %
51	+80 %	+80 %	+80 %	100 %	+80 %
52	+100 %	+100 %	+100 %	100 %	+100 %
Note that: the number of *Moina* provided was constant across all the treatments during transition					
Up 92 DAH	>2000 *Moina* per individual + Microdiet (ranging from 1.2 g to -1.5 g for each tank)				

completely replaced. However, the treatment that received co-feeding diet MD at larval stage was maintained on the same diet in the second experiment and since it performed better than other diets it was regarded as a control. Sampling was done every after 10 days of culture for one month to record the weights and the lengths. The experiment was terminated at 92 DAH (last day of sampling) when the whole body of each larval was observed to have completely been covered with scales. During the whole period for experiments 1 (from 6 DAH to 92 DAH) about 5 fish were observed of their scalation process every sampling to clearly identify the period of larvae transition into juvenile stage. Observations were made using light microscope (model Leica DM 500, Made by Microsystems Switzerland Ltd).

2.2. Experimental study 2: ontogenetic enzyme activity and regulation in larvae fed combination (MD)

The ontogeny and regulation of enzyme activity of amylase, protease and lipase enzymes were determined to explain the growth changes during larval development for the best performing combination diet (MD) in experimental study 1 (MD was selected based on pre- experiment trials). Enzyme activity helped identify the maturation digestive competent period for break down and utilization of starch, proteins and lipids by larvae. This experiment was done concurrently with experimental study 1 (Phase I) under the same experimental set up and conditions for uniformity of growth results for the combination diet MD. In

this experiment (Experimental study 2), 10 aquaria of 55 l capacity each were treated with the combined *Moina* and microdiet (MD). The aquaria were randomly stocked with between 100 and 200 larvae each. Rearing conditions and feeding regimes were maintained similar to experiment 1a until 45 DAH. Larvae were randomly sampled in equal proportion from the 10 tanks before the morning feeding, anaesthetized with an over dose of clove oil and weighed to achieve at least 0.5 g (constituting between 50–150 individuals depending on the age and weight). Larvae were immediately put in 20 mL plastic bottles and preserved in nitrogen filled tank (-80°C). Samples were collected every 2 days from 1 DAH up to 8 DAH, then every 3 days up to 35 DAH, and there after every 5 days up to 45 DAH. The samples were transferred to the bioscience lab at the National Crop Research Institute at Namulonge (NaCRI) for analysis of enzymatic profiles.

To prepare the enzyme extract, fish samples earlier stored at −80 °C (from experiment 1b) were placed in 50 mL falcon tubes containing 15 mls of phosphate buffered saline (PBS) to stabilize the reaction mixture and prevent possible hydrolytic activities (Dawson et al., 1986). The samples were homogenized using an ultrasonic homogenizer (model 150 V/T-Biologics Inc) at −20 °C. The homogenate was then centrifuged at 10,000 rpm for 15 min. The supernatant was decanted and stored at -20°C for later enzyme activity analyses.

Enzyme activity for amylase, protease and lipase were determined following a method modified from Kimura and Robyt (1995); Sigma-Aldrich (1999) and Sugihara et al. (1991) respectively. The modified procedures are elaborated in Aruho (2018). Total protein in sample extracts was determined so as to calculate specific activity per milligram protein. Protein was essayed based on Bradford (1976) method. A standard curve was made using bovine serum albumin (BSA) of 0.1−0.6 mg/mL. 240 μL of Bradford reagent was mixed to 10 μL of homogenate; 200 μL from each aliquot was placed in spectrophotometer and absorbance values were read off at 595 nm.

2.3. Experimental study 3: green water and larvae culture

In another set of experiment to determine whether green water had any direct effect on growth of *B. altianalis* larvae, 75 larvae of average weight 3.0 ± 1.0 mg (6 DAH) each were randomly distributed into 6 glass tanks of 55 L. Three tanks were given green cells (Green water cultures- mixed microalgae-dominated by *Chlorella vulugaris*) estimated at 6.3×10^6 cells per litre. The rest of the tanks were used as control. Larvae in all the tanks were fed with *Moina* + microdiet (DM) following the same procedure as used in experiment 1. Temperature was kept at 27 ± 1°C by thermostatic heaters. Green water was added daily in tanks after cleaning them of feaces or uneaten feed at 8.00 h and 16.00 h and before feeding the larvae. The tanks were kept in a greenhouse and received 12 h of day light and 12 h of darkness. The weights and the lengths of the larvae were recorded at every sampling done after 14 days for a period of 42 days (6 DAH to 48 DAH).

2.4. Experimental study 4: growth performance of larvae co-fed microdiet from outdoor concrete tanks

Based on the results obtained from a procedure of larval growth experiments in indoor aquaria (experimental study 1-Phase 1and II), an outdoor experiment to evaluate growth performance for fingerling production of *B. altianalis* larvae nursed in a semi natural controlled environment was conducted at ARDC-Kajjansi (N° 13′19.182″. E 32° 32′4.9092″). Three concrete tanks (100 m^2, 1 m deep) were thoroughly cleaned and disinfected with sodium hypochlorite (NaOCl). They were fertilized with application of NPK (nitrogen, phosphorous & potassium) at a rate of 80 g/1000 L to encourage growth of both phytoplankton and Zooplankton. When the tanks were green, 3000 larvae (14.0 ± 1.82 mg; 15 DAH) raised indoor on MD diet were randomly and equally distributed into the three replicate tanks. Water inlet and the outlet were tightly screened with a 250 μm mesh to avoid entrance of unwanted natural predators and loss of zooplanktons. The tanks were covered with mosquito nets to prevent birds and other insects falling into the tanks. Feed supplementation was done 3 times a day by feeding the larvae with DF diet (57 % CP) at 8.00 h, 12.00 h and 17.00 h. Sampling to record the wet weight was conducted only twice on the 45 DAH (after one month) and on 75 DAH (second month) when the larvae were observed to have metarmophosised into the Juveniles. Two hundred larvae were weighed at each sampling. Larvae were counted to ascertain the mortalities. The water quality (Ammonia and Dissolved oxygen) was maintained by flashing in the water at slow flow rate of 10 s per litre for one hour at 1.00 h, 5.00 h, 12.00 h and 7.00 h. Average ammonia was 0.13 ± 0.06 ppm and Dissolved oxygen was 4.6 ± 1.2 mg/l. Ammonia was monitored using LaMotte Fresh water aquaculture kit (Code 6665-02-CC-) while the dissolved Oxygen was monitored by OxyGuad Handy Polaris 2 Portabe DO Metre.

2.5. Preparation of live feeds and decapsulation of Artemia cysts

Green water culture was prepared as a feed for *Moina micrura*, one of the live feeds that was used to feed the larvae either alone (MO) or in combination with a dry feed (MD). Preparation of the green water cultures and enrichment of *Moina micrara* followed a procedure described by Aruho (2018). *Artemia* nauplii and decapsulated cysts were prepared following modified procedure by Sorgeloos (1980) and Spotte (1992) respectively, and described in detail by Aruho (2018).

2.6. Growth parameters

The weight gain percentage, specific growth rates (SGR), survival and condition factors were calculated using the following equations:-

FW-IW

$$\text{(i)}\quad \text{Wg}\% = \frac{\text{FW} - \text{IW}}{\text{IW}} \times 100.$$

$$\text{(ii)}\quad \text{SGR}\%\ \text{per day} = \frac{\text{In FW} - \text{In IW}}{\text{n}} \times 100.$$

$$\text{(iii)}\quad \text{Survival}(\%) = \frac{\text{FN}}{\text{TN}} \times 100.$$

$$\text{(iv)}\quad \text{K} = \frac{\text{W}}{\text{L}^3} \times 100$$

$$\text{Weight-Length relationship} = \text{W} = \text{aL}^{\text{b}} \qquad \text{(v)}$$

Where a and b are coefficients, L is the total length (cm), and W is the wet weight in g; FW is the Final Weight; IW is Initial weight; In is the natural log; SGR is the Specific Growth Rate, Wg is Weight gain, n is the number of culture days; K is Fulton's condition factor (Froese, 2006); FN is Final Number; TN is Total number of stocked fish

2.7. Data analysis

An interactive effect between sampling period and the treatment means (weight) due to larval diets was analysed using repeated measures Analysis of Variances (ANOVA). Statistical significance between treatment means at each sampling level (period) was determined using Duncan's test in one way ANOVA. Relationships between weights and total lengths of larvae and juveniles were calculated by linear regression analysis. Chi-square statistical test (X^2) was used to compare the larval survival and mortality frequencies using cross-tabulation (contingency table) analysis technique. Student's *t*-test was used to analyze the weight differences between green water fed larva and non green water fed larvae. Statistical analyses were all performed using IBM SPSS Statistics for Windows (Version 22.0. Armonk, NY: IBM Corp, 2013) at 95 % confidence level.

Table 3
Mean values (±Standard Error) of larval body weight fed different diets at $P < 0.05$.

DAH	DAF	MO (mg)	MD (mg)	HA (mg)	DA (mg)	DF (mg)
13	7	3.30 ± 0.10^{a}	14.93 ± 0.36^{c}	3.21 ± 0.08^{a}	12.87 ± 0.21^{b}	13.31 ± 0.25^{b}
20	14	8.63 ± 0.30^{a}	31.05 ± 0.58^{e}	14.80 ± 0.35^{b}	24.16 ± 0.32^{d}	$20.99 + 0.42^{c}$
28	21	19.17 ± 0.53^{a}	63.64 ± 1.08^{e}	24.81 ± 0.53^{b}	40.30 ± 1.13^{d}	32.52 ± 1.00^{c}
34	28	37.24 ± 0.75^{a}	87.59 ± 1.45^{e}	42.07 ± 0.66^{b}	70.04 ± 1.72^{d}	55.61 ± 1.65^{c}
41	35	44.66 ± 0.93^{a}	110.65 ± 1.70^{e}	55.07 ± 0.86^{b}	102.98 ± 1.97^{d}	65.40 ± 1.73^{c}
48	42	73.67 ± 1.48^{b}	152.05 ± 2.51^{e}	60.35 ± 0.83^{a}	141.14 ± 2.43^{d}	87.30 ± 2.63^{c}

Mean values with different superscripts show significant differences.

Table 4
F-tests for all the diets at each sampling at $P < 0.05$.

DAH	DAF	F	Level of significance	Degree of freedom (treatments, subjects)	No. of fish N
13	7	146.19	<0.001	4, 10	678
20	14	65.97	<0.001	4, 10	678
28	21	62.72	<0.001	4, 10	678
34	28	18.52	<0.001	4, 10	678
42	35	75.80	<0.001	4, 10	678
48	42	58.45	<0.005	4, 10	678

3. Results

3.1. Effect of live and microdiets on growth performance of larvae and juveniles (experimental study 1 (Phase I & II)

The interaction between treatments (diets) and sampling period was significant throughout the culture period ($P < 0.001$). That is, there was significant weight increase due to the diet effect after every sampling for each diet. At first sampling, 13 DAH (7 DAF), larvae fed diet MD attained significantly higher average body weight BW than the larvae fed all other diets ($F_{4, 10} = 146.19$, $P < 0.001$) followed by larvae fed diets DA, DF, MO and HA in that order. There was no statistical significance in average BW between larvae fed diets DA and DF ($P > 0.05$). The larvae fed diets MO and HA attained the least BW and there was no statistical difference between them ($P > 0.05$). In all the subsequent samplings done at 20, 27, 34, 41 and 48 DAH, BWs of the larvae on all diets were significantly different from each other (Table 3; Table 4). Larvae fed diet MD maintained a significantly better growth performance than all other diets followed by DA, DF, MO and HA in that order (Table 3). In spite of the significant differences in BW between larvae fed diets MD and DA their means were closer and bigger than the rest of the treatments. Significant variations in the final specific growth rates SGR ($F_{4, 10} = 45.66$, $P < 0.001$), weight gain W ($F_{4, 10} = 58.85$, $P < 0.001$) and condition factor K ($F_{4, 10} = 60.25$, $P < 0.001$) were observed for all diets with a better growth performance recorded for diet MD followed by DA, DF, MO and HA (Table 5).

At the time of termination of experimental study 1(Phase I), larval percentage survival was high in all the treatments (between 77.7 % and 92.1 %). There were no significant differences in survival among larvae fed diets MO, MD, HA, and DA ($P > 0.05$) but survival of larvae fed diet DF was significantly lower than for all other diets ($X^2 = 21.173$, df = 4, p = 0.0001). However, mortalities were comparatively higher in larvae fed diets MO, MD, HA and DA before the 24 DAH (18 DAF) than those larvae fed the same diets by 45 DAH (35 DAF) (Fig. 1). Mortality remained relatively constant for larvae fed diet DF throughout the experimental.

In the second phase of the experimental study 1(Phase II), where all the treatments received a combination of *Moina* + *microdiet* (MD), the interaction between treatment (diets) and sampling period was significant throughout the culture period ($P < 0.001$). No significant differences in final average body weight BW were noted between larvae in

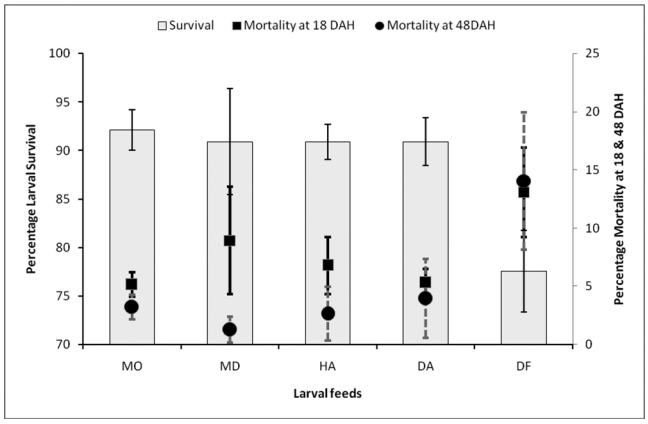

Fig. 1. Mean percentage survival at 48 DAH and percentage mortalities of *B. altianalis* Larvae at 24 DAH and 45 DAH. Bars are shown as mean ± Standard Deviation.

Table 5
Growth parameters of *B. altianalis* fed on different diets (Mean ± SE).

Growth Parameters	MO	MD	HA	DA	DF
Experiment 1(Phase I); 48 DAH (42 DAF)					
Initial Weight (mg)	3.00 ± 1.00	3.00 ± 1.00	3.00 ± 1.00	3.00 ± 1.00	3.00 ± 1.00
Final weight (mg)	73.67 ± 1.48^{b}	152.05 ± 2.51^{e}	60.35 ± 0.83^{a}	141.14 ± 2.43^{a}	87.30 ± 2.63^{c}
Weight gain W %	2355.80 ± 49.33^{b}	4968.40 ± 83.74^{e}	1912.26 ± 27.50^{c}	4604.69 ± 81.00^{d}	2809.88 ± 87.59^{c}
SGR%	3.28 ± 0.02^{b}	4.06 ± 0.01^{e}	3.10 ± 0.01^{a}	3.96 ± 0.02^{d}	$3.41 \pm 0.0.04^{c}$
Final Condition factor K	0.70 ± 0.01^{b}	0.88 ± 0.01^{e}	0.64 ± 0.01^{a}	0.85 ± 0.01^{d}	0.72 ± 0.01^{c}
Survival%	93.3^{b}	93.0^{b}	92.9^{b}	92.9^{b}	82.9^{a}
Experiment 1 (Phase II); 92 DAH (86 DAF)					
Initial Weight (mg)	73.67 ± 1.48^{b}	152.05 ± 2.51^{e}	60.35 ± 0.83^{a}	141.14 ± 2.43^{a}	87.30 ± 2.63^{c}
Final weight (mg)	355.47 ± 6.08^{a}	500.20 ± 11.80^{b}	361.01 ± 5.85^{a}	510.13 ± 11.93^{b}	354.69 ± 12.60^{a}
Weight gain W %	382.514 ± 8.26^{d}	228.85 ± 7.76^{a}	498.19 ± 9.69^{e}	261.43 ± 8.45^{b}	306.29 ± 14.43^{c}
SGR%	1.53 ± 0.02^{d}	1.14 ± 0.02^{a}	1.75 ± 0.02^{c}	1.23 ± 0.02^{b}	1.30 ± 0.04^{c}
Final Condition Factor K	0.89 ± 0.02^{a}	0.87 ± 0.01^{a}	0.85 ± 0.02^{a}	0.85 ± 0.01^{a}	0.89 ± 0.01^{a}
Weight length relation (power curves)	$y = 0.002x^{4.022}$; $r^2 = 0.969$	$y = 0.004x^{3.503}$; $r^2 = 0.959$	$y = 0.002x^{3.974}$; $r^2 = 0.963$	$y = 0.004x^{3.465}$; $r^2 = 0.979$	$y = 0.003x^{3.660}$; $r^2 = 0.968$

Different superscripts across the rows indicate significant differences.

Table 6
Statistical significance between treatment mean weights at $P = 0.05$ from 62 DAH to 92 DAH. (Mean ± Standard Error SE).

DAH	DAF	MO (mg)	MD (mg)	HA(mg)	DA (mg)	DF (mg)
62	56	170.00 ± 3.39[a]	263.36 ± 4.30[b]	167.82 ± 2.36[a]	279.04 ± 5.13[b]	179.71 ± 5.63[a]
72	66	251.18 ± 3.77[a]	355.33 ± 6.44[b]	237.81 ± 3.46[a]	373.11 ± 6.97[b]	236.9 ± 6.41[a]
82	76	325.17 ± 3.28[a]	427.30 ± 8.22[b]	315.90 ± 4.21[a]	443.22 ± 9.69[b]	305.96 ± 12.60[a]
92	86	355.47 ± 6.08[a]	500.20 ± 11.80[b]	361.01 ± 5.85[a]	510.13 ± 11.93[b]	354.69 ± 12.60[a]

Treatments with different superscripts along the same row are significantly different.

treatments MD and DA ($P > 0.05$) throughout the experimental period. Similarly, no significant differences in BW were noted among the larvae in treatments MO, DF and HA ($P > 0.05$). However, larvae in MD and DA treatments maintained a significantly higher final average BW than larvae in MO, DF, and HA treatments (Table 6). Significant differences among all treatments were observed in larval weight gain W ($F_{4,\ 10} = 28.98$, $P < 0.001$) and specific growth rates SGR ($F_{4,\ 10} = 32.36$, $P < 0.001$). No significant differences in condition factor were observed among all the treatments ($P > 0.05$). In spite of the higher average BW of larvae noted in DM and DA treatments, the weight gain W and SGR were much higher for larvae in treatments MO, HA and DF, signifying a previously and comparatively much deeper growth depression and therefore a higher compensatory growth performance than larvae on diets MD and DF (Table 5). There was no death recorded during the transition period of introducing new diet MD (co-feeding) between 48 DAH and 52 DAH until when the experiment was terminated at 92 DAH. The power curve equations relating the standard weight and the total length showed a very strong correlation relationship r^2 of greater than 95 % for each diet and all showed positive allometric growth in all the treatments (Table 5). The power curve equations for diet DM and DA; and those for MO and HA were generally similar showing a similar growth trajectory for the diets.

3.2. Ontogeny of enzyme activity and larval development of the best diet MD (6 DAH to 45 DAH; experimental study 2)

The mean average growth was 152.05 ± 3.51 SE from the 10 experimental tanks. No amylase activity was detected before and until the mouth opening on day 5. The amylase activity surfaced on the 6 DAH (8 ± 2 U/mg Standard deviation) when feeding started and increased sharply from that day reaching the peak at 14 DAH (23 ± 4 U/mg) and then reduced to 17 DAH (11 ± 2 U/mg) and gradually continued to reduce up to 45 DAH (Fig. 2). The alkaline proteases were detected at the beginning of exogenous feeding, increased sharply to 17 DAH (100 ± 9 U/mg) and dropped low to 20 DAH (40 ± 4 U/mg) and gradually until the end of the experiment period (Fig. 3). Lipase activity increased to the highest peak at 17 DAH (11 ± 0.5 U/mL) then slightly reduced to 20 DAH (8 ± 0.9 U/mL) and rose again to 28 DAH (9.5 ± 0.3 U/mL) before it gradually declined at 40 DAH (3 ± 0.30 U/mL) (Fig. 4).

3.3. Green water (microalgae) experiment

A significant difference in larval growth was observed between the treatment that was given algae (green water) and that of the clear water at 20 DAH ($t_{374} = -5.67$, $P < 0.0001$). Conversely, no significant differences were observed in larval weight between the treatments in all the subsequent samplings at 34 and 48 DAH ($P > 0.05$) (Table 7). No significant differences in SGR, W and percentage survival were observed between the two treatments ($P > 0.05$) (Table 7).

3.4. Nursing larvae in outdoor green water concrete tanks

Final average BW was 1112 ± 42.70 mg (±SE) at 75 DAH after two months of stocking. The BW at 15 DAH was 7.24 ± 0.34 and 250.77 ± 7.1 at 45 DAH. The SGR for the whole experimental period was 3.149 ± 0.02 % (± SE). While weight gain W was 7843.32 ± 169.44 %. Survival

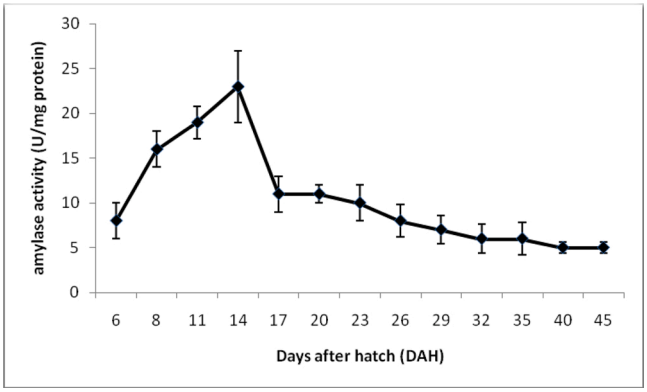

Fig. 2. Variation of Amylase activity with Days after hatch (DAH) in *B. altianalis* larvae (n = 3). Bars are shown as mean ± Standard Error.

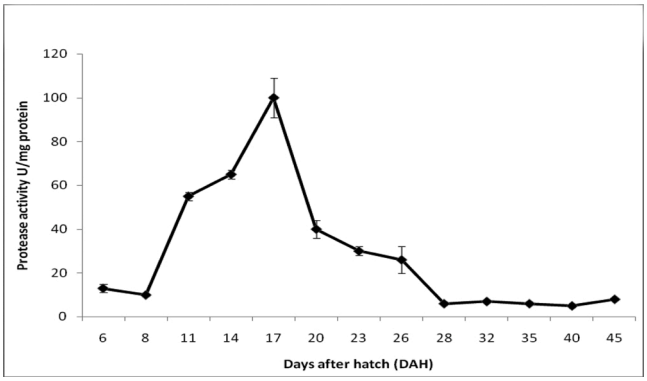

Fig. 3. Variation of protease activity with Days after hatch (DAH) in *B. altianalis* larvae (n = 3). Bars are shown as mean ± Standard Error.

was 89.23 % at 45 DAH and 95.3 % between 45 DAH and 75 DAH. Water temperature was 24 ± 1.2°C, dissolved oxygen was recorded at 6.3 ± 1.5 mg L^{-1} (± standard deviation) and the Ammonia was ≤ 0.01 ppm.

3.5. Scale formation (larval transition to juvenile)

Throughout the experimental period from 6 DAH to 92 (for experiment 1, Phase I & II), scales were first observed in the larvae fed MD and DA diets forming around the neck and a long lateral region at 41 DAH when the larvae were 110.65 ± 1.70 mg (±SE) and 102.98 ± 1.97 mg (±SE) respectively. The scalation increased rapidly and by 75 DAH (427.0 ± 4.1 mg; ±SE) they had covered an estimated 80 % of the whole body (Fig. 5). For the larvae that were stocked in outdoor concrete tanks for further nursing (experimental study 3) the scales had completely covered the whole body by the second sampling at 75 DAH (1112 ± 42.70 mg; ±SE).

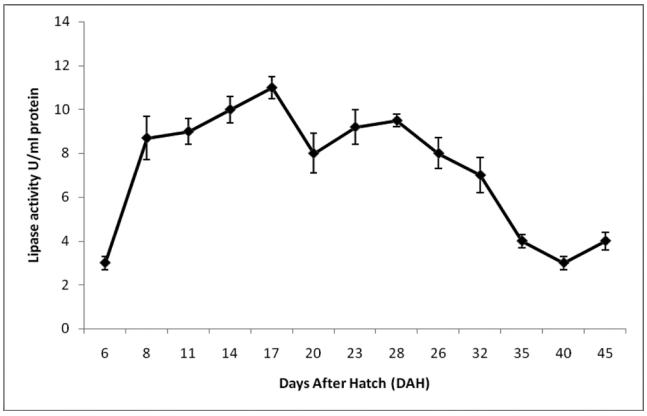

Fig. 4. Variation of lipase activity with Days after hatch (DAH) in *B. altianalis* larvae (n = 3). Bars are shown as mean ± Standard Error.

Table 7
Mean values of growth parameters between green water and clear water treatments.

DAF	DAH	Green water (mg)	Clear water (mg)
14	20	35.33 ± 0.57[a]	39.95 ± 0.58[b]
28	34	89.84 ± 2.00[a]	92.22 ± 1.00[a]
42	48	147.19 ± 2.98[a]	148.59 ± 2.90[a]
Final weight (mg)		147.19 ± 2.98[a]	148.59 ± 2.98[a]
Specific growth rates SGR%		3.989793 ± 0.02[a]	3.994740 ± 0.02[a]
Weight gain W %		4806.4030 ± 99.32[a]	4852.9411 ± 96.36[a]
Survival %		78.0[a]	79.3[a]

Treatments with different superscripts along the same row are significantly different at $P < 0.05$.

Fig. 5. Scale formation. a) Scales forming round the neck and along the lateral region of *B. alitianalis* larvae metamorphosising into Juveniles at 57 DAH (257 mg, 3.0 TL). b) The animal has transited into juvenile and the whole body is almost covered by scales at 75 DAH (427 mg, 3.9 TL). The juvenile clearly resembles an adult fish. c) Magnified section of the skin of young fish showing formed scales. The scales gave a characteristic glittering colouration of an adult *B. altianalis* Mg X 100.

4. Discussion

Larval growth and survival in captivity are influenced by a multiplicity of factors ranging from nutritional quality, maturation and digestive capability of larvae, management and control of key physico-chemical water parameters, hygiene in the hatchery (Braum, 1967; Delince et al., 1987) and often genetic quality of the species (Lorenzen et al., 2012). In this study larval growth and survival were found to be significantly affected by the diets and the age at which the diets were offered. Results from experimental study 1(Phase 1) showed that in *B. altianalis* the specific growth rates (SGR), weight gain (W), condition factor (CF) and final average body weight (BW) for larvae co-fed *Moina* + microdiet (MD) performed better than all other diets. This was followed by larvae fed decapsulated *Artemia* (DA), the microdiet alone DF, *Moina* (MO) and the hatched *Artemia* (HA). These results agree with those obtained from some other cultured species including, *Aristichthys nobilis* (Fermin and Recometa, 1988), *Clarias Batrachus* (Giri et al., 2002), *Migurnus anguillicaudatus* (Wang et al., 2008), *M. anguillicaudatus* (Wang et al., 2009), *Oncorhynchus mykiss* (Akbary et al., 2010) and *Osteobrama belangen* (Ramesh et al., 2014). These authors reported that larvae fed combination diet resulted in better growth and survival than those fed microdiet or live food alone. The current results also concurred with the same studies in *A. nobbilis* and *O. Mykisss* in which larvae fed microdiet alone performed better than those fed live diet. However, the microdiet given to the *O. belangen* larvae performed poorly with high larvae mortalities than *Moina* (Ramesh et al., 2014). With a better final BW for larvae fed diet DF (87.30 ± 2.63 mg) than MO (73.67 ± 1.48 mg) and HA (60.35 ± 0.83 mg), this study confirmed that *B. altianalis* larvae could be directly weaned to a microdiet though this was not the best diet since significant mortalities were recorded compared to other diets. Variations in nutrient composition for different microdiets will influence how fast the digestive capability of the larvae develops ably to digest and assimilate the microdiet for better larval growth (Cahu and Zambonino-Infante, 2001; Cara et al., 2003). The analysis on DF diet used in this study showed that this commercial diet was well formulated with minimum nutritive requirements for better larvae growth and survival. Hence larvae performance was consequently limited by other factors such as sufficient endogenous enzymes for digestion.

The good performance of diet MD in *B. altianalis* was attributed to a contribution of nutrients from *Moina* and microdiet. Live feeds are said to contribute enzymes for either self digestion or facilitate direct breakdown of microdiets (Dabrowski, 1984; Kolkovski et al., 1993). Some authors suggest that enzyme contribution from the live feed is very small and that co-factors carried by live prey stimulate more larval pancreatic secretions that facilitate maturation of the digestive process to efficiently digest and assimilate the microdiets (Cahu and Zambonino-Infante, 2001; Liu et al., 2012). Other workers suggest that, visual and chemical stimulation by the live prey facilitate capture and ingestion of microdiet for quick growth (Canavate and Fernandez-Dıaz, 1999). The current study results agree with the later assertions because the larvae fed exclusively on microdiet performed better than the live diets. The active feeding of larvae observed more in co-fed treatments preceded stimulation of enzyme co-factors. Nevertheless, co-feeding maximized nutritional benefits from live and microdiets improving growth and survival of *B. altianalis*. The study findings recommend inclusion of a co-feeding strategy in larval rearing protocol.

The larval ontogenetic enzyme pattern indicating an early increase in activity, followed by a sharp decline for larvae fed combination diet, is a characteristic of larvae maturation and development manifested in many other species at various stages (Zambonino-Infante et al., 2008). All enzymes were detected at the time of exogenous feeding and their initial increase was attributed to the development and size increase of the pancreas as was observed in several cultured cyprinids (Chakrabarti et al., 2006; Chakrabarti and Rathore, 2010; Farhoudi et al., 2013). Early increase in amylase and then its sharp decline is said to be genetically programmed in larvae development (Peres et al., 1996; Cahu and Zambonino-Infante, 2001). The decline of other pancreatic enzymes as the larvae grew did not reflect enzyme reduction but suggested a normal increase in protein tissue as the fish gains weight (Cara et al., 2003; Zambonino-Infante et al., 2008). The evolution of enzyme activity pattern in developing larvae relates the digestive maturation with food assimilation mechanisms, and this helps to indentify the period when

the larvae are competent enough to effectively digest and assimilate the microdiet (Zambonino-Infante et al., 2008; Gisbert et al., 2009). Thus, at a time when the pancreatic enzyme activity began to decline, the larvae digestive structure developed an efficient digestive mechanism that was attributed to gradual increase of enterocyte enzymatic activity as a result of increased gut microvilli structural development (Kolkovski, 2001; Gisbert et al., 2009). This observation was confirmed by increased clarity and size of microvilli and the larval intestinal folds by the 15 DAH (Aruho et al., 2019). It can be inferred that the earliest *B. altianalis* larvae fed combination diet can reach competent digestive maturation stage with minimal mortalities and better BW was at 14 DAH, 20 DAH and 17 DAH for amylase, protease and lipase respectively. To be explicit, by the third week of maturation after hatch, *B. altianalis* larvae were easily able to adapt to dietary changes i.e. digest and assimilate complex diets with inevitable manipulations or possible complete replacement of live diets with microdiets and with very minimal mortalities. The attainment of digestive maturation level in common carp larvae was able to facilitate complete diet replacements in a related study (Farhoudi et al., 2013). Diet manipulation and or complete replacement of live preys by microdiets is an inevitable strategy in massive seed production to lower the production costs and increase survival (Kolkovski, 2001; Conceicao et al., 2010).

The gradual decline of enzyme activity coincided with the reduction of larvae mortality in a combination diet. Mortality was slightly high for larvae fed diet MD before 24 DAH but declined further than other diets by 48 DAH confirming the possibility of attaining a gradual maturation and stabilization of the digestive capability of the larvae earlier than other diets. In spite of the fact that larvae fed exclusively with microdiet DF performed better than those fed live diets, survival was significantly lower than other diets. Most studies attribute the poor performance of the microdiets to limitations in enzyme production to facilitate larval digestion of the microdiet, due to rudimentary digestive system (Segner et al., 1993; Kolkovski, 2001; Ramesh et al., 2014). However, the survival reported in such studies has been notably too low compared to the 77.6 % obtained for *B. altianalis* in the current study. It can be deduced that whereas there are sufficient nutrients for larval uptake from the microdiet; there could still be an insufficient mechanism for self released enzymes to appropriately breakdown and utilized the processed microdiets. This mechanism seemed to select against the small larvae in aquaria because most of the dead larvae were small. It is hypothesized that the feed could have chronically stressed their digestive system rendering them physiologically unable to adjust and produce sufficient enzyme to digest the microdiet compared to bigger sized larvae. The stress factor could be justified by the fact that larvae fed live diets had comparatively less mortalities than microdiet. The role of stress effect by microdiets could further be investigated in this species.

In experimental study 1(Phase II), no larvae mortality was recorded throughout the growth period. The absence of mortalities coincided with a rise in larval weight at the start of the Phase II experiment and was attributed to gut coiling reported in previous study on the same species at 35 DAH (Aruho et al., 2019). In some species, gut coiling resulted into increased surface area over which food absorption occurred (Gisbert et al., 2009; Ruan et al., 2012). This change also marked the beginning of scalation characterizing a transition process into juvenile stage. Compensatory growth was observed in all treatments but the juveniles in treatment DA (initially fed decapsulated *Artemia* at larval stage) grew comparatively faster to the same rate with those in control treatment MD than other treatments. Earlier in experiment 1 (Phase I), larvae fed decapsulated *Artemia* had exhibited growth performance close to those fed MD diet and therefore reached a digestive maturation level, much earlier than larvae fed diets MO, HA and DF before all were treated to co-feeding in Phase II. Decapsulated *Artemia* has good and well composed nutrients with sufficient energy for larvae growth (Leger et al., 1986). Therefore, the introduction of co-feeding (*Moina* + microdiet) provided a quick impulse for further (or better) growth. The current study concurred with larval studies in *Carassius auratus* (Kaiser et al., 2003) and in *Solea solea* (Leger et al., 1986) where decapsulated *Artemia* performed better than *Artemia* nauplii. Good larval performance on decapsulated *Artemia* was also recorded in *Cyprinus carpio* (Vanhaecke et al., 1990), *Leuciscus cephalus* L (Shiri-Harzevili et al., 2003) and *Clarias gariepinus* (Olurin and Oluwo, 2010). From this study it can be stated that the decapsulated *Artemia* provided a better alternative diet for growth of *B. altianalis* larvae to reach a digestive maturation level earlier than *Artemia* nauplii. The decapsulated *Artemia* contain more energy than other forms of *Artemia* nauplii (Vanhaecke et al., 1983; Bengtson et al., 1991) and therefore *B. altianalis* larvae easily consolidated this energy in decapsulated form as starter feed than the nauplii. The challenge with *Artemia* diets in Africa is the high prohibitive cost and availability issues which hinder fingerling production.

The initial larval growth limitations observed in treatments MO, HA and DF in experiment 1(Phase I) was attributed to inadequate nutrients in diet or enzymes for digestion. When co-feeding was introduced Phase II, larvae maximized available nutrients and grew comparatively faster than those in MD and DA. But all larvae in all indoor aquaria tanks soon reached their normal physiological potential under. This could be the reason why the condition factor was notably the same across all treatments. Therefore, the nutritional composition of diets was no longer a limiting factor but perhaps other factors that included environmental stress and or stocking densities (Basiao et al., 1996; Ali et al., 2003). This result had a significant implication in growth of *B. altianalis* larvae because initial diet provided to the larvae will consequently influence growth and survival in juvenile stages. High larval growth rates will confer to juveniles' size advantages to accessing limited resources, evading and escaping predators (Leggett and Deblois, 1994). In tropical marine fishes high larval growth rates were associated with survival during metamorphosis to juvenile stages (McCormick and Hoey, 2004). With *B. altianalis* it is inevitable to maintain an initial feeding protocol of co-feeding and or using decapsulated *Artemia* to facilitate early larvae maturation and subsequent quick growth transition to juvenile stages.

Manipulation of environment can largely influence compensatory growth (Ali et al., 2003). Hence when 15 DAH larvae were transferred and nursed in outdoor green water concrete tanks (in experiment 4) the final BW of 1112 ± 2.43 mg was attained at 75 DAH compared to 355.33 ± 6.44 mg fed combination diet in indoor aquaria within the same period. In this study (experiment 3), indoor green water experiment did not suggest a direct role of green water as reported in other studies by Reitan et al. (1997); Bengtson et al. (1999) and Sanaye et al. (2014) on other species. Green water only improved the nutritional value of *Moina* as also stated by Fermin and Recometa (1988) in bighead carp, *Aristichthys nobilis*. In a more stable semi natural system such as well fertilized bigger nursing ponds or tanks microalgae could offer a characteristic and complex environment that supports robust growth and survival than in smaller confined indoor aquaria tanks. Studies on larvae of Atlantic halibut *Hipoglossus hippoglosuss* (Skjermo and Vadstein, 1993) and herring *Clupea harengus* (Hansen et al., 1992) indicated that the addition of green algae to rearing tanks influenced microbial balance mechanism in rearing water and with in the gut system of the larvae and consequently affected the growth and survival of larvae in these species. In grass carp *Ctenopharyngodon idella* and bighead *Aristichthys nobilis* outdoor facilities provided better growth than indoor facilities (Opuszynski et al., 1985). This result is similar to what was obtained in this study when two weeks old larvae were nursed in outdoor facility; and the mechanisms for better growth may have been largely facilitated by complex microalgae interactional role. Further investigations are recommended to identify the age when *B. altianalis* juveniles can directly use and incorporate algae diets into their digestive systems and also the possibility of microbial role as a result of green water technique that influences the survival of larvae or juveniles among other factors.

5. Conclusion

In conclusion, several studies define the larvae transition age to juvenile stages as the period of metamorphosis until the larvae acquire all adult features (Jones et al., 1978; Kendall et al., 1984). Based on the evidence adduced from this study of scale formation process, growth parameters and ontogenetic control of enzymatic activity coupled with structural ontogenetic histological development of the digestive system, it can be concluded that the larvae of *B. altianalis* transformed into juveniles between the age of 48 (150 mg) and 75 DAH (427 mg). But these were also influenced by the type of diet provided. The study affirmed that the larvae of *B. altianalis* are vulnerable in outdoor nursing environments especially before two weeks after hatch but higher chance of their survival was increased with the transformation of the larvae into juveniles. With better controlled environment of free parasites and good water quality rich in live feed, outdoor nursing of larvae offers faster growth rates and survivals of *B. altianalis* with supplementation of a well nutrient balanced microdiet. This is a feasible and cheaper option for its production in large quantities. This study suggests that stocking *B. altianalis* juveniles in much bigger culture systems such as ponds is preferable during or after larvae has fully transformed into juveniles (after two months).

Author contributions

The idea was conceived by Cassius Aruho the author, who conducted growth experiments, analyzed and drafted the paper. This was done under the supervision of Dr. Martin Sserwadda, Prof. Fred Bugenyi and Prof. Justus Rutaisire who guided and helped refine the paper. Dr. Martin also participated in analysis of fatty acids profiles at the University of Ghent in Belgium. Dr. Ephraim Nuwamanya and Dr. Walakira were instrumental in technical designing and guiding the protocol for analysis of enzyme profiles of the developing larvae. Professor Russell J. Borski provided technical guidance on the paper structure particularly on the methodology and guided internal review process.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aqrep.2020.100441.

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