Development of Diet for Larval *Catla catla, Labeo rohita* and *Cirrhinus mrigala*

Huma Nasrullah[1], Muhammad Ashraf [1*]

Abstract

Larval Catla catla, Labeo rohita and Cirrhinus mrigala depend on live food. Quality and quantity of live food is always difficult to meet the requirements of larval fish. From the last several decades nutritionists have been working to replace live food with convenient artificial feed but still its replacement has not been accomplished. Therefore, current studies were an attempt to formulate and develop artificial feed for larval stages of these species. Each species was divided into two groups. In Group 1, each species fed on natural food and served as control and the second group exclusively fed on artificial feed and termed as treatment group- the outcomes of which were unknown. Both groups were fed on live food (Brachionus calyciflorus) and artificial feed (40% C.P) respectively for 15 days in three independent trials for all the three species tested. Studies were conducted in 3×2×2 ft. glass aquaria with water holding capacity of 255 L. In each trial 100 larvae were randomly stocked in each replicate aquaria of each treatment including control. During these studies Labeo rohita larvae fed on artificial feed gained maximum weight of 52.7mg, significantly higher than its counterpart. When larval growth of all the three species was compared the following trend emerged Labeo rohita 52.7 mg >Cirrhinus mrigala 44.6 mg >Catla catla 32 mg. Nonetheless larvae of all the three species when fed on natural food grew in the following order Labeo rohita 46.7 mg >Cirrhinus mrigala 35.6 mg >Catla catla 26.4 mg. Like weight Labeo rohita fry displayed maximum survival of 71% when fed on artificial. Survival of larval Cirrihinus mrigala was 61% and that of Catla catla was 52% the lowest. In general artificial feed performed better than natural food (control groups).

***Keywords**: Labeo rohita, Catla catla, Cirrhinus mrigala, larval fish, artificial feed, growth, live food.*

Introduction

Quality and quantity of appropriate feed is still a major constraint in successful rearing of early fry. Presently mass production of fish fry under controlled

[1] University Veterinary and Animal Sciences, Ravi Campus Pattoki, Main Office Lahore
Email:mawarraich17@gmail.com

https://doi.org/10.58932/MULH0018

conditions heavily depends on the provision of live planktonic food due to its poorly developed intestinal tract lacking appropriate capability of digesting complex inert diets. The importance of live food in fry and larval rearing has been thoroughly investigated and well documented (Ajah, 1997, 1998; Ajah & Holzlöhner, 1996; Hagiwara et al., 1997; Ovie et al., 1993).

The unicellular algae, *Chlorella vulgaris*, rotifer, *Brachionus plicatilis*, and brine shrimp, *Artemia salina* are currently widely in use for larval stages of fish. Though larvae are fed on live organisms such as algae and micro crustaceans but technically it is not only difficult but in some cases impossible too (Gabaudan 1984). Most of the time quality and quantity of live foods do not match the nutrient requirements of the larvae (Sorgeloos 1980; Watanabe et al. 1983). Moreover the culture of live food organisms such as *Daphnia*, *Artemia* and *Brachionus* or their collection from natural sources is also problematic and risky because can bring with them pathogens or parasites to the hatchery Uys & Hecht 1985).

Carps fry are fed on both living as well as nonliving foods. Live food includes zoo plankton like brine shrimp (Artemia), nauplii, rotifers, and cladocerans etc. Food supplements fulfill additional nutritional demand of fish fry for rapid growth and good and healthy. So there is a need to develop inert diets for early and later stages of life of Indian Major Carps.

Carp culture has been practiced in India for the past 3-5 decades. East, west, and southern states of India are prominent in this practice. Most of the farmers use oilseed cake (groundnut oil cake) and rice bran mixture for juvenile and adult stages of fish (Mohanty, 2006). Therefore to overcome such problems and make the aquaculture operations viable it is important to replace the live foods by cost effective and easily available artificial diet. Artificial diets have several advantages. The feed and feed-stuff from which they are prepared can be stored. Their size and composition can be adjusted to the exact requirement of fish. Pathogen or parasite-free diets can be prepared on mass scale by proper sterilization ensuring regular supply (Uys & Hecht 1985).

Therefore artificial feed formulation is urgently required and has a huge demand globally. Fishes have four prominent developmental stages namely spawn, fry, fingerling, and young fish. Feed and feeding habit vary at each stage. Particularly feed formulation and feed development is the least focused area due to flaws in nutrient requirements of this stage (Sirdeshmukh & Shembekar, 2019) that need to be optimized. In Indian Major Carps hatchlings do not eat any exogenous food. They depend on their yolk sac and move vertically. On absorption of yolk sac fry start

eating exogenous food. At this stage they can move in all directions. The larval stages of all the three Indian carps feed on phyto and zooplanktons until they attain fingerling stage when they adapt adult feeding habit. At this stage they grow very fast and quickly transits from one stage to the next stage.

Fish feed formulation is one of the most important area to work in aquaculture (2019) due to its huge demand all over the world. Fishes have four prominent stages and each stage demands different types of feeds both qualitatively and quantitatively. Information on nutrient requirements of larval, fry and fingerling stages has not been yet fully comprehended that is essential for competent feed formulation. Therefore current studies were initiated on species specific feed formulations for larval stages of three Indian major carps to reduce their feeding costs and to improve growth and survival because studies are limited on artificial feed formulation early stages of Indian major craps due to their reliance on natural food. Therefore objectives of the present studies were 1; Indoor culture of algae(*Chlorella vulgaris*) and *Brachionus calyciflorus*), and 2; Intensive rearing of larval *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* on natural and artificial diets

Materials and Methods

Study site

The experiment was conducted at fish breeding facility of Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus Pattoki, Punjab, Pakistan.

Experimental design

In current studies experiment was planned and designed following two-group simple randomized design (Kothari, 2004). Studies were conducted in 3×2×2 ft. glass aquaria with water holding capacity of 255 L. Group 1 served as control and second as experimental. Both groups had two replicates. Feeding studies were planned on larval *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala.* In each trial 100 larvae of each relevant fish species were randomly stocked in each replicate aquaria of each treatment including control. Whole study was split into three trials, and each trial consisted of single fish species. Group 1 in each trial was fed on natural food and group 2 (experimental group) on artificial feed.

Preparation Of Larval Food

Algae Culture (*Chlorella vulgaris*)

Algae culture was split into three distinct stages; starter, intermediate and mass scale culture following Ashraf et al. (2011). Culture conditions were, temperature 18-29 ^{0}C, pH 7.0-9.0, light intensity 2500-5000 lux, photoperiod 14.0 hours, and DO 3-8 ppm.

Rotifer (*Brachionus calyciflorus*) culture

Rotifers were cultured in plastic jars at small scale and at mass scale in fiberglass tanks following Ashraf et al.(2010). Presence of male in culture or cyst production was an indication of sexual reproduction and maturity of culture. Rotifers were totally harvested at this stage, counted by Sedgwick Rafter counting cell and fed to larval fish.

Fig. 1: *Female Rotifer*

Fig. 2: *Male Rotifer*

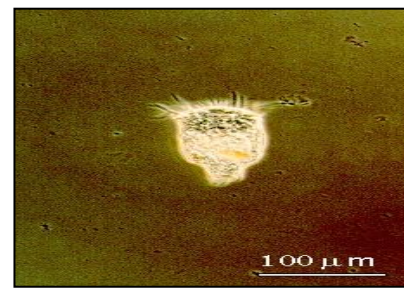

Fig. 3*:* *Rotifer Cyst*

Composition of Artificial Feed

Table 1: *Composition of Artificial Feed Used for Intensive Rearing of Larval Catla catla, Labeo rohita and Cirrhinus mrigala*

Ingredient	Wt. of each feed ingredient used(g)	CP level %	CP contribution from each ingredient
Fish meal	100	50	25
Cotton Seed meal	150	34	50
Canola meal	150	34	51
Soybean Meal	200	47	94
Guar Meal	86	35	30.1
Rice polish	313	12	37.56
Nutrimix	10	----	
Total	1000 g		300g (30%)

Experimental protocol

Fry were stocked in glass aquaria of size (3×2×2 ft.) with 255 liter water holding capacity. Before stocking all the aquaria were well cleaned with tube well water and then disinfected with 400 ppm $KMnO_4$ solution. Aquaria were filled with fresh tap water up to 1.5 ft. level.

On the 4^{th} day of hatching 100 fry of each fish species were randomly placed in each replicate glass aquaria of control and experimental group. A random sample of 5 fish was taken from the original stock for preliminary weight and length measurements. Fish was raised in hatchery water under low level constant aeration. The fluorescent lights present in the laboratory provided necessary light at an ambient photoperiod. Water temperatures ranged from 24-26 ^{0}C and pH was 7.75-8.3 throughout the feeding studies.

Every morning before the first feeding, the jars were cleaned by a flanged plastic tube. Gentle rotation of the tube scraped the bottoms of the jars. Uneaten food, fecal material and extraneous matter was removed from the jars. Any dead fish were removed and counted daily. One third of the total jar volume was removed and replaced with fresh tap water. The fry were fed daily at 100% of their total wet biomass. All the diets were dispensed by hand and stirred in water for a short time. The daily ration was weighed and given out in three equal installments at 10, 14 and 18 hours. The feed was offered in progressively increasing amounts at the rate of 3% of the starting ration every day from the day of commencement to the day of

termination of the experiment. The trials were continued for 2 weeks. At the end of experiment, the water was completely drained off and the fish were harvested on a 150 µm net suspended in 2-phenoxy ethanol. The narcotized animals were blotted dry, weighed by an electronic balance to the nearest 0.1 mg and measured with a caliper to the nearest 0.1mm.

Percent weight gain = final weight- initial weight ×100/ initial weight

SGR% = log of final weight – log of initial weight x 100/ no of days

FCR = feed fed on dry weight basis/weight gained on wet weight basis

Statistical Analysis

One way analysis of variance followed by Duncan's Multiple Range Test was used to evaluate the statistical significance of the treatment differences. Differences between the treatment means were considered significant at $P<0.05$ level.

Results

Small scale Algae culture

In small scale culture, algae sample was inoculated in the media at the density of 1.05 million cells /ml. Algae gained peak value of 3.57 million cells/ml at temperature of 21.9^{0}C, pH 8.7, and DO 4.91 mg/l. In intermediate scale culture ($\geq$ 2L - <100L) algae sample was inoculated with algae sample of 3.09 million cells/ml. It gained peak value of 5.21 million cells/ml at temperature 26^{0}C, pH 8.6, DO 4.01 mg/l. In large scale culture($\geq$200L) the media was inoculated with an algae sample having density of 4.82 million cells/ml. The algae gained peak value of 9.01 million cells/ml at 29.1^{0}C, pH 7.9, DO 5.02 mg/l(Table # 2).

Table 2: *DO Temperature and pH Measured on Daily Basis for Small, Intermediate and Large Scale Algae Culture.*

Parameter/Month	2nd week of May	Last week of May	Mid of June
Small scale culture			
DO mg/L	4.80	4.67	4.91
Temperature ^{0}C	22.3	22	21.9
pH	8.4	8.1	8.7
Algal density(million cells/ml)	2.41	2.94	3.57

Intermediate scale culture			
DO mg/L	3.8	3.9	4.01
Temperature ^{0}C	25.3	25.4	26.0
pH	8.0	8.4	8.6
Agal density(million cells/ml)	2.61	3.42	5.21
Large scale culture			
DO mg/L	4.01	5.5	5.02
Temperature ^{0}C	28.1	28.3	29.1
pH	8.01	8	7.9
Algal density (million cells /ml)	7.6	8.23	9.01

Rotifer density

Stocking density was 1-2 rotifer female / ml. At the outset of the studies stocking density of rotifers was 142 individuals /ml and the peak value was 10050 rotifer/ml(Table # 3).

Table 3: *Rotifer Density Observed at Various Times of the Summer Months*

Date	**No. of rotifers/ml**
March	142
1st week of April	361
2nd week of April	780
Last week of April	1554
1st week of May	3350
2nd week of May	4964
Last week of May	6880
1st week of June	8100
3rd week of June	10050

Table 4: *DO Temperature and pH Measured on Daily Basis for Larvae of Catla Catla Fed on Natural Food Fed on Artificial Feed?*

Parameter/month	**25th May**	**1st June**	**6th June**
	Natural food		
DO mg/L	3.93	4.05	3.97
Temperature ^{0}C	28.1	27.1	27.7
pH	7.2	7.79	7.3
	Artificial feed		
DO mg/L	3.71	3.93	4.1
Temperature ^{0}C	27.9	27.5	27.9
pH	7.5	7.41	7.4

Table 5: *DO, Temperature and pH Measured on Daily Basis for Larvae of Cirrhinus Mrigala Fed on Natural and Artificial Feed?*

Parameter/month	1st June	6th June	11th June
Natural food			
DO mg/L	4.22	3.83	3.43
Temperature ^{0}C	29.3	31.3	30.02
pH	7.97	8.17	7.92
Artificial feed			
DO mg/L	3.78	2.93	3.21
Temperature ^{0}C	28.95	31.7	30.0
pH	7.61	7.81	7.7

Table 6: *DO, Temperature and pH Measured on Daily Basis for Larvae of Labeo Rohita Fed on Natural and Artificial Feed?*

Parameter/month	15th June	21st June	26th June
Natural food			
DO mg/L	4.01	4.25	4.31
Temperature ^{0}C	30.91	32.01	32.0
pH	7.9	7.6	7.6
Artificial feed			
DO mg/L	4.37	4.71	7.9
Temperature ^{0}C	31.0	32.3	7.6
pH	7.5	32.61	7.6

Table 7: *Growth Data of Cirrhinus mrigala*

Parameters	Treated group	Control group
Initial weight(mg)	9.4 ± 0.02^a	9.4 ± 0.02^a
Final weight(mg)	54 ± 1.1^a	45 ± 1.3^b
Gain in weight(mg)	44.6 ± 1.7^a	35.6 ± 0.5^b
Percentage weight gain	474.47 ± 12.4^a	378.723 ± 10.3^b
SGR %	0.75 ± 0.01^a	0.68 ± 0.01^b
FCR	3.16 ± 0.03^a	3.96 ± 0.02^b
Initial length(mm)	7 ± 0.01^a	7 ± 0.01^a
Final length(mm)	19 ± 1.2^a	17 ± 1.0^a

Increase in length(mm)	12 ±0.7[a]	10± 1.5[a]
Survival %	67±1.3[a]	53±1.2[b]

Table 8: *Growth Data of Catla catla*

Parameters	**Treated group**	**Control group**
Initial wt.(mg)	8±0.01 [a]	8 ±0.01[a]
Final wt.(mg)	40 ± 1.2[a]	34.4±1.3[b]
Gain in wt.	32 ±1.3[a]	26.4 ±0.9[b]
Percentage wt. gain(mg)	400 ±5.3[a]	330±4.7[b]
SGR %	0.44±0.02[a]	0.38±0.03[b]
FCR	3.75±0.5[a]	4.54±0.4[b]
Initial length (mm)	6 ±0.5[a]	6±0.3[a]
Final length (mm)	15.2±1.2[a]	13.5± 0.7[a]
Increase in length (mm)	9.2± 1.2[a]	7.5±1.1[a]
Survival % (mm)	52±2.5[a]	44±1.9

Table 9: *Growth Data of Labeo rohita*

Parameters	**Treated group**	**Control group**
Initial wt.(mg)	9.3±0.02[a]	9.3±0.03[a]
Final wt.(mg)	62±1.9[a]	56±1.4[b]
Gain in wt.(mg)	52.7±1.3[a]	46.7± 0.8[b]
Percentage wt. gain(%)	566.67 ±10.3[a]	502.15±9.7[b]
SGR %	0.82±0.03[a]	0.77±0.01[b]
FCR	2.64±0.06[a]	2.98±0.03[b]
Initial length(mm)	8 ±0.9[a]	8 ±0.7[a]
Final length(mm)	21±1.8[a]	19 ±1.6[a]
Increase in length(mm)	13± 1.5[a]	11± 1.3[a]
Survival %	71±1.3[a]	64±2.1[b]

Discussion and Conclusion

At small scale culture algae gained peak value of 3.57 million cells/ml at temperature of 21.9^{0}C, pH 8.7, and DO 4.91 mg/l. In intermediate scale culture algae gained peak value of 5.21 million cells/ml, and in large scale culture algae gained peak value of 9.01 million cells/ml. The temperature, DO, and pH ranged from 21.9^{0}C to 29^{0}C, 4.01 ppm to 5.02 ppm, and 7.9 to 8.7 respectively. Algae cells/ml gradually increased with the rise in temperature as well as with expansion in algal culture set-up. Maximum rotifer density was 10050 individuals/ml.

Algal depends on the incident light (photons flux density (PFD) with given angular distribution and spectrum. The incoming light instigates various compounds present in water dissolved inorganic carbon, dissolved oxygen, and h mineral nutrients which in turn promote the synthesis of various compounds that support growth of algae. Presence of these compounds requires optimum levels of water quality parameters such as pH, temperature with absence of contaminants. All these factors in desirable range can initiate and optimize the photosynthetic activity of algae. These variables are almost uniform despite species of algae cultured and location of its setup. Maintaining optimal growth conditions to achieve maximum scaling up is really difficult task.

Tian et al.(2024) reported nitrogen removal efficiencies of *Desmodesmus* sp. at temperatures of 20 °C, 25 °C, and 30 °C. There was significant increase in removal efficiency of NH_4-N, NO_3-N, and NO_2-N with rise in temperature that agrees with our findings that showed gradual increase in productivity when temperature increased. It was also true for rotifers as well as for the treatment of municipal wastewater by microalgal bacteria consortium (Kant Bhattia et al., 2022). Elevated temperatures boost the metabolic rate in microalgae, thereby improving nutrient removal efficiency when the temperature remains below the ideal level for the growth of microalgae (Xu et al., 2019). Therefore, increased temperatures play a significant role in improving nitrogen elimination and absorption ultimately increasing algal production

The initial weight of larval *Cirrhinus mrigala* was same (9.4mg) in both control and treated group (Table 10). The final weight of larvae was 54mg and 45mg in treated and control group respectively (Table 10). Weight gain in treated group was significantly higher than control group. Final weight gain (44.6 mg) in fish fed on artificial feed was significantly higher than control group (35.6 mg). Similarly percentage weight was also significantly higher (475.47%) in artificial feed group than control (378.7%). Specific growth rate (SGR) and feed conversion ratios (FCR)

followed the same trend and values were 0.75, 0.68, 3.16 and 3.96 in treated and control groups respectively and remained significant between each other.

Larvae *Labeo rohita* fed on artificial feed displayed maximum survival (71 %). Survival in other fish species ranked *Cirrihinus mrigal* 61% >*Catla catla* 52%; live feed: *Labeo rohita* 64% >*Cirrihinus mrigala* 53% >*Catla catla* 44% the lowest.

Though feeds in all the trials were well pulverized and matched the size of the mouth but still larvae hesitated to take in the feed particles. Nonetheless after continuous feeding and provision of feed particle in suspending condition enticed larvae to initiate to accept feed particle though with slow intial response. After the lapse of one week larvae fully accepted and started to take in the feed particles. Fish feed's perception depends on availability, appearance (Roch et al., 2020), particle size (Hutchings, 2011), and taste (Kasapis, 2009). Commercial feeds typically contain high-quality fish meals and have a relatively good smell that attracts post larvae to the feed hence larvae accept them immediately. Hence fish meal was used in this feed at quite higher concentration to attract larvae. Feed particles were considerably reduced to match larval mouth size.

Hence acceptance was good which motivated larvae to take more food that supported its growth better than control group. Hansika et al.(2024) reported higher survival and weight gain in larval *Catla* fed with high protein artificial feeds. Performance of the same larvae remained poor when fed with low protein feeds. Our studies are quite close to Hansika et al.(2024) and Sirdeshmukh et a.(2019) and further confirm the positive role of quality artificial feeds in rearing of post larval Indian major carps. Previously Shahi et al.(2022) have the similar findings where they observed significantly higher growth and survival in *Cyprinus carpio* when fed with combined feeding schedule with more inclination to artificial feeds. Therefore artificial feeds are much better than natural foods if formulated and processed properly.

References:

Ashraf, M., Javaid, M., Rashid, T., Ayub, M., Zafar, A., Ali, S., & Naeem, M. (2011). Replacement of expensive pure nutritive media with low cost commercial fertilizers for mass culture of freshwater algae, *Chlorella vulgaris*. *International Journal of Agriculture and Biology, 13*, 484–490.

Ashraf, M., Ullah, S., Rashid, T., Ayub, M., Bhatti, E. M., Ali, S., & Javid, M. (2010). Optimization of indoor production of freshwater rotifer, *Brachionus calyciflorus*, b: Feeding studies. *Pakistan Journal of Nutrition, 9*(6), 582–588.

Božidar, S. R., Marko, B. S., Zoran, Z. M., & Vesna, D. P. (2011). Histological methods in the assessment of different feed effects on liver and intestine of fish. *Agricultural Sciences*, 56(1), 87–100.

Francis, O. A. (2006). Culture of the freshwater rotifer, *Brachionus calyciflorus*, and its application in fish larviculture technology. *African Journal of Biotechnology*, 5(7), 536–541.

Hansika, R. K. N., Withanage, M. P., Fouzi, M. N. M., Muneeb, M. M., & Rahmathullah, M. (2024). Development of an efficient fish feed for *Catla catla* post larvae to enhance the survival rates of nursery stages by using available raw materials. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. *Veterinary Medicine, 81*(1),14–15.

Kant Bhatia, S., Ahuja, V., Chandel, N., Mehariya, S., Kumar, P., Vinayak, V., Saratale, G. D., Raj, T., Kim, S.-H., & Yang, Y.-H. (2022). An overview on microalgal-bacterial granular consortia for resource recovery and wastewater treatment. *Bioresource Technology*, 351, 127028.

Kasapis, S. (2009). Developing minced fish products of improved eating quality: An interplay of instrumental and sensory texture. *International Journal of Food Properties*, 12(1), 11–26.

Kothari, C. R. (2004). Research design. In *Research methodology: Methods and techniques* (2nd rev. ed., pp. 31–54). New Age International Publishers.

Lubzen, E., Gibson, O., Zmora, O., & Sukenik, S. (1995). Potential advantages of frozen algae (*Nannochloropsis sp.)* for rotifer (*Brachionus plicatilis*) culture. *Aquaculture*, 133(3–4), 295–309.

Marina, M., Camargo, P., Cláudia, B., & Martinez, R. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotropical Ichthyology, 5*(3), 327–336.

Richard, S. S. (1981). A general approach to the culture of planktonic rotifers. *Canadian Journal of Fisheries and Aquatic Sciences, 38*(6), 721–724.

Roch, S., Friedrich, C., & Brinker, A. (2020). Uptake routes of microplastics in fishes: Practical and theoretical approaches to test existing theories. *Scientific Reports, 10*(1), 3896.

Shahi, S., Singh, G., Mehta, P., & Dahiya, T. (2022). Effect of different dietary sources on the rearing of Amur carp (*Cyprinus carpio haematopterus*). *Biological Forum – An International Journal, 14*(4), 889–894.

Sirdeshmukh, U. P., & Shembekar, V. S. (2019). Feed formulation and optimization of fish feed for different developmental stages of Indian major carps from spawn to marketable size fish stage. *Journal of Emerging Technologies and Innovative Research (JETIR), 6*(6), 654–659.

Tian, X., Lin, X., Xie, Q., Liu, J., & Luo, L. (2024). Effects of temperature and light on microalgal growth and nutrient removal in turtle aquaculture wastewater. *Biology, 13*(11), 901.

Xu, K., Zou, X., Wen, H., Xue, Y., Qu, Y., & Li, Y. (2019). Effects of multi-temperature regimes on cultivation of microalgae in municipal wastewater to simultaneously remove nutrients and produce biomass. *Applied Microbiology and Biotechnology, 103*, 8255–8265.

Yoshimura, K., Hagiwara, A., Yoshimatsu, T., & Kitajima, C. (2011). Culture technology of marine rotifers and the implications for intensive culture of marine fish in Japan. *Marine and Freshwater Research, 47*(2), 217–222.

Winkler, M., Hemschemeier, A., Gotor, C., Melis, A., & Happe, T. (2002). [Fe]-hydrogenases in green algae: Photo-fermentation and hydrogen evolution under sulfur deprivation. *International Journal of Hydrogen Energy, 27*, 1431–1439.