DEVELOPMENT OF LARVAL DIET FOR *Ompok bimaculatus* A HIGH VALUE FISH OF REGIONAL IMPORTANCE

FINAL PROJECT REPORT

DURATION: 01/04/2017 - 31/03/2020

ICAR-Central Institute of Freshwater Aquaculture
(ISO 9001:2015 Certified Institute)
(Indian Council of Agricultural Research)
RRC,Rahara, 700118,West Bengal

ANNEXURE -VI

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

CHECKLIST FOR SUBMISSION OF FINAL RESEARCH PROJECT REPORT (RPP-III)

(For Guidelines Refer ANNEXURE – XI (F))

1. Institute Project Code: I-95-G

2.Investigators as approved in RPP-I, If any change attach IRC proceedings:

Principal Investigator	CC-PI	Co-PI
Dr. B.N.Paul		Dr. S Adhikar Dr.P.P.Chakrabarti Dr. N Mandal Mr. A Das Dr. K Ghosh (Burdwan University)

3.Any change in objectives and activities No
(If yes, attach IRC proceedings)

	Date of Start & Date of Completion (Actual). If any extension granted enclose IRC proceedings		Yes	
	Whether all objectives met		Yes	
	All activities completed		Yes	
	Salient achievements/major recommendations included		Yes	
	Annual Progress Reports (RPP-II) submitted	1st Year	Yes	
		2nd Year	Yes	
		3rd Year	Yes	
		nth year		No
	Reprint of each of publication attached		Yes	

	Action for further pursuit of obtained results indicated	Yes	No
	Report presented in Divisional seminar (enclose proceedings & action taken report)	Yes	No
	Report presented in Institute seminar (enclose proceedings & action taken report)	Yes	
	IRC number in which the project was adopted	IRC No: I-95-G	
	Any other Information		

4.Signature:

Project Leader	Co-PI	Co-PI	Co-PI	Co-PI
(Dr. B.N. Paul)	(P.P. Chakrabarti)	(Dr. S. Adhikari)	(Dr. R.N. Mandal)	(Mr. A. Das)

Co-PI

(Dr. K. Ghosh)

HOD/PD/I/c.

ANNEXURE – VII

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
FINAL RESEARCH PROJECT REPORT (RPP- III)
(For Guidelines Refer ANNEXURE – XI(G))

1. Institute Project Code : I-95-G
2. Project Title: **Development of Larval diet for *Ompok bimaculatus*, a high- valued fish of regional importance**
3. Key Words: *Ompok bimaculatus,*Ontogeny, larvae, egg, enzyme,larval diet and nutrient composition
4. (a) Name of the Lead Institute : ICAR-Central Institute of Freshwater Aquaculture,
5. (b) Name of Division/ Regional Center/ Section: Regional Research Center, ICAR-CIFA, Rahara

   (a) Name of the Collaborating Institute(s): University of Burdwan

   (b) Name of Division/ Regional Center/ Section of Collaborating Institute(s)

   Dept. of Zoology, Burdwan Rajbati, Raiganj, Burdwan, West Bengal 713104
6. Project Team(Name(s) and designation of PI, CC-PI and all project Co-PIs, with time spent)

Sl. No.	Name, designation and institute	Status in the project (PI/CC-PI/ Co-PI)	Time to be spent (%)	Work components assigned to individual scientist
1	Dr. B.N.Paul	PI	50	Planning, Nutrient analysis, evaluation of larval feed, data analysis and reporting
2.	Dr.P.P.Chakrabarti	Co-PI	25	Brood stock development of high value species
2	Dr. S Adhikari	Co- PI	25	Water quality parameters and reporting.
3	Dr. R.N Mandal	Co-PI	25	Production of Live food organisms ,data analysis and reporting

4	Mr. A Das	Co-PI	25	Production of larvae, Evaluation of larval feed, data analysis and reporting
Collaborating University: University of Burdwan, West Bengal				
5.	Dr. K. Ghosh	Co-PI	25	Ontogeny study of larvae and enzyme analysis.

7. Priority Area: Aquaculture Diversification.

8. Project Duration: Date of Start – **01.04.2017**

Date of Completion – **31.03.2020**

9. a. **Objectives**

1. To study nutrient composition of egg and different stages of larvae

2. To study the ontogeny of larval development.

3. To formulate larval feed and evaluation

b**. Practical utility**

Ompok bimaculatus, popularly known as the 'butter catfish', is a freshwater teleost species native to the South-East Asia. It is piscivorous as well as carnivorous fish. Due to its air-breathing nature, it could become a very good candidate species for stocking small, shallow seasonal ponds and tanks which are oxygen-deficient and could grow to required marketable size in just 6months. Over the years, the natural occurrence of this fish species has been depleted due to some anthropogenic activities and hence it has been listed under Near Threatened (NT) category of IUCN Red List (2010). This fish has a high commercial value and preferred in Eastern and North-Eastern India. *O. bimaculatus* was also declared as the State Fish of Tripura in the year 2007.The captive breeding of *O. bimaculatus* has opened a new road map for successful aquaculture of the species. During its metamorphosis, larval rearing is a big challenge. The larval stage is a critical stage in fish life cycle that necessitates an appropriate exogenous nutrition once the embryonic yolk is used up. Research has been carried out over the few decades to reduce the period over which live pray must be used, using better understanding of larval behavior and physiology and

improvement in microparticle formulation. The success of larval rearing is greatly influenced by first feeding regimes and the nutritional quality of starter diets. Thus to domesticate the species larval rearing is a bottleneck, especially the larval feed. Thus the present proposal is aimed at development of larval feed for *O. bimaculatus* so that the mortality will be reduced and more stocking material would be available for successful growout of *O. bimaculatus.*

Keeping in view of the above concept an attempt has been taken up to domesticate *Ompok bimaculatus* in terms of its understanding of larval nutrition and feed development.

Collaborating University: University of Burdwan

10. Final Report on the Project (materials and methods used, results and discussion, objective wise achievements and conclusions)

1. **Material** : *Ompok bimaculatus*

2. **Techniques/Methodology** :

3. The larval feed development for the *O. bimaculatus* is critical as the species requires exogenous nutrition once the embryonic yolk is exhausted. The development of exogenous larval feed is very essential at this stage. The study of ontogeny of larvae will give a guideline ragarding the enzyme status and metamorphosis of its digestive system. Co-feeding strategy to be evolved

for proper feeding of the larvae with natural and formulated feed. Further the exogenous formulated feed will be developed for the larvae.

4. Sample Analysis:

5. The samples were collected as per the method of Sankar (2010).The nutrient analysis of brood fish, egg and larvae and feed samples were done as per AOAC (2005). Water quality was studied as per APHA (2005).

6. Fatty acid analysis:

7. Extraction of pooled samples for fatty acid analysis was done as per Folch *et al*. (1957). Preparation of Fatty acid Methyl Esters (FAME) was done as per Metcalfe *et al.* (1966). The prepared samples were quantified by injecting into Gas Chromatograph (GC) (Perkin Elmer; CLARUS 480). GC operating software 'Total Chrom' was used and identification of individual Fatty Acid was done by comparison of retention time to those of standards (SUPELCO, Cat.No. 47885-U). Data were subjected to statistical analysis as per Snedecor and Cochran (1994) and the least significance difference (LSD) was used for comparison of the mean values.

Gas Chromatograph (GC) (Perkin Elmer; CLARUS 480).

8.

9. Enzyme assay: Digestive enzymes was assayed as per Reitman and Frankel (1957).

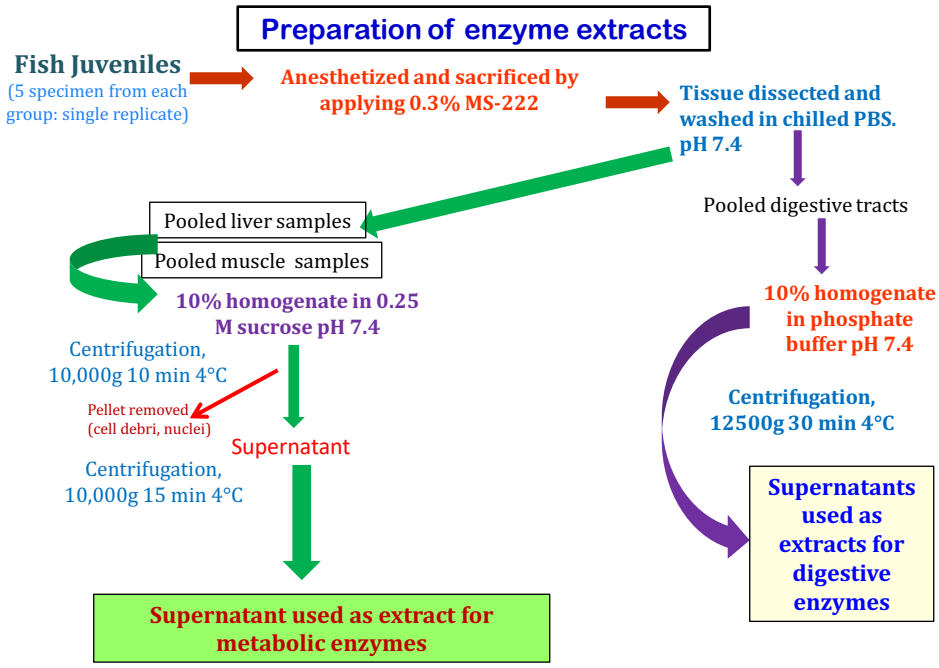
1. Protease, amylase and lipase activities was measured after Walter (1984), Bernfeld (1955) and Bier (1955), respectively
2. Estimation of trypsin and chymotrypsin activity according to Erlanger et al. (1961)
3. Apart from digestive enzymes, the following metabolic enzymes were studied as key enzymes of the major metabolic pathways:

Glucose 6 phosphate dehydrogenase (De Moss, 1955), cytosolic NADP malate dehydrogenase (Hsu and Lardy, 1967), mitochondrial NAD malate dehydrogenase (Englard and Siegel, 1969), alanine

transaminase (Reitman and Frankel,1957), aspartate transaminase (Reitman and Frankel, 1957), glucose-6-phosphatase (Marjoric, 1964), fructose- 1,6-bisphosphatase (Freeland and Harper, 1959) and hexokinase (Tranulis et al., 1996)

4. Changes in the profile of proteolytic enzymes through ontogeny was studied through separation and characterization of proteases by substrate SDS-PAGE after Garcıa-Carreno et al. (1993).

Average live weight gain (%), specific growth rate (SGR: % day−1), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU%) were worked out as per (Steffens, 1989).



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Objective1. To study nutrient composition of egg and different stages of larvae

Nutrient composition Egg, Brood fish and Larvae

The proximate and fatty acid composition of *Ompok bimaculatus* (pabda) brood fish, egg and larvae was assayed to have an insight of the nutrient metamorphosis during early life of the species and vis-à-vis parental nutrient composition. The collected brood fish, eggs and larvae were analyzed for proximate and fatty acid composition.

The brood fish and its egg were collected from ICAR-Central Institute of Freshwater Aquaculture, Kalyani field station. Soon after hatching the *O. bimaculatus* larvae were also collected from Kalyani Field Station. The collected samples of brood fish, egg and larvae of ompok were iced immediately and sent for analysis. Sampling procedure and sample preparation was done as per the standard methodology of Sankar *et al.* (2010).

The proximate composition of Pabda brood, egg and larvae are presented in Table 1. The data revealed that the crude protein, fat and ash content of brood fish, egg and larvae were 14.4±0.07, 25.90±0.20, 12.23±0.88; 1.06±0.07, 0.64±0.15, 0.42±0.04 and 2.33±0.05, 1.61±0.10 and 1.79±0.04 (%) respectively.. The studies revealed that the crude protein content was significantly ($P<0.01$) higher in pabda egg however moisture, crude lipid and total ash (%) was significantly ($P>0.01$) lower pabda egg. The result of the study also concluded that the moisture, crude lipid and total ash (%) was significantly ($P<0.01$) higher in pabda brood.

Table 1: Proximate composition of Brood pabda, egg and larvae

Particulars	Brood Pabda	Pabda Egg	Pabda Larvae
Moisture	80.09 ± 0.64^{b}	61.84 ± 1.40^{a}	82.02 ± 0.62^{b}
Crude Protein	14.44 ± 0.07^{a}	25.90 ± 0.20^{b}	12.23 ± 0.88^{a}
Crude Fat	1.06 ± 0.07^{b}	0.64 ± 0.15^{a}	1.61 ± 0.10^{a}
Total Ash	2.33 ± 0.05^{b}	1.61 ± 0.10^{a}	1.79 ± 0.04^{a}

Data presented as Mean± S.E. Superscripts ^{ab} in a row differs significantly ($P<0.01$)

Table 2: Fatty acid (% of total fatty acid) composition of Pabda brood, egg and larvae

Particulars	Pabda brood	Pabda Egg	Pabda Larvae
Butyric Acid	0.02 ± 0.01^{a}	0.01 ± 0.001^{a}	2.08 ± 0.05^{b}
Myristic acid (C14:0)	0.12 ± 0.01^{a}	0.22 ± 0.03^{a}	4.03 ± 0.57^{b}
Palmitic acid (C16:0)	87.81 ± 1.53^{b}	66.15 ± 0.77^{a}	22.52 ± 0.64^{a}
Arachidic acid (C20:0)	0.2 ± 0.03^{a}	0.05 ± 0.001^{a}	2.31 ± 0.12^{b}
Others	0.33 ± 0.02^{a}	9.59 ± 0.19^{b}	17.85 ± 0.52^{c}
ΣSFA	88.48 ± 0.66^{c}	76.02 ± 0.98^{b}	48.6 ± 0.66^{a}
Pentadecanoic acid (C15:1)	0.62 ± 0.05^{b}	0.4 ± 0.001^{a}	1.29 ± 0.015^{c}
Oleic acid (C18:1n9c)	0.61 ± 0.001^{a}	12.08 ± 0.09^{c}	4.29 ± 0.08^{b}
Elaidic acid (C18:1n9t)	6.03 ± 0.02^{a}	ND	11.02 ± 0.1^{b}
Others	0.41 ± 0.02^{a}	1.43 ± 0.07^{b}	1.42 ± 0.02^{b}
ΣMUFA	7.67 ± 0.12^{a}	13.91 ± 0.24^{b}	18.02 ± 0.02^{c}
Linolelaidic acid (C18:2n6t)	0.02 ± 0.01^{a}	0.06 ± 0.01^{a}	7.03 ± 0.08^{b}
Linoleic acid (C18:2n6c)	1.89 ± 0.09^{b}	4.25 ± 0.09^{c}	0.47 ± 0.01^{a}
ϒ-Linolenic acid (C18:3n6)	0.05 ± 0.01^{a}	0.43 ± 0.02^{b}	0.56 ± 0.02^{c}
α-Linolenic acid (C18:3n3)	0.1 ± 0.01^{a}	0.21 ± 0.01^{b}	1.58 ± 0.045^{a}
Eicosadienoic acid (C20:2)	0.28 ± 0.006^{a}	0.51 ± 0.01^{b}	1.4 ± 0.01^{c}
Eicosatrienoic acid (C20:3n6)	0.64 ± 0.02^{a}	1.68 ± 0.02^{b}	11.17 ± 0.06^{c}
Eicosatrienoic acid (C20: 3n3)	ND	0.05 ± 0.01^{a}	0.32 ± 0.02^{b}
Arachidonic acid (C20:4n6)	ND	0.07±0.01	ND
EPA (C20:5n3)	0.73 ± 0.03^{b}	0.23 ± 0.01^{a}	2.71 ± 0.01^{c}
DHA(C22:6n3)	0.8 ± 0.02^{a}	2.56 ± 0.04^{b}	6.87 ± 0.03^{c}
ΣPUFA	4.52 ± 0.03^{a}	10.05 ± 0.02^{b}	33.35 ± 0.40^{c}
ω 3	1.63 ± 0.06^{a}	3.05 ± 0.04^{b}	11.5 ± 0.02^{c}
ω 6	2.6 ± 0.07^{a}	6.49 ± 0.08^{b}	19.23 ± 0.01^{c}

Data presented as Mean± S.E. Superscripts ^{abc} in a row differs significantly ($P<0.05$)

ND: Not detected

Haul of pabda brood fish at Kalyani Field Station

Brood fish (*O. bimaculatus*)

***O.bimaculatus* brood fish**

The fatty acid profile obtained by GC analysis are presented in Table 2. The fatty acid composition revealed that the saturated fatty acid (SFA) content (% of total fatty acid) of brood pabda, eggs and larvae were 88.48%; 76.04% and 48.60%. Among the MUFA, oleic acid and elaidic acid was significantly ($P<0.05$) higher in pabda larvae. The poly unsaturated fatty acid (PUFA) content (% of total fatty acid) of brood pabda, eggs and larvae were 4.5%; 10.05% and 33.35% respectively in brood fish, egg and larva. The EPA and DHA contents were 2.71% and 6.87% in larve; which was significantly ($P<0.05$) higher in comparison to brood fish (EPA-0.73% and DHA-0.80%) and egg (EPA-0.23% and DHA-2.56%). Among PUFA, total ω-3 content was 1.63, 3.05 and 11.5 (%) respectively in pabda brood, egg and larvae

The studies regarding nutritional characterization of brood fish, eggs and larvae of *Ompok bimaculatus* revealed that crude protein content was higher in egg whereas crude fat and ash content was higher in brood fish. However, PUFA, EPA and DHA content was higher in larvae.

Objective 2. To study the ontogeny of larval development.

The Ontogeny of *Ompok bimaculatus* was studied with the hatched larvae at ICAR-CIFA, Kalyani field station.he ontogeny and enzyme study was done at Burdwan University, which is the collaborating University in the project.The digestive tract of *O. bimaculatus* comprised of a straight tube dorsally attached to the yolk sac. Table 3. Describes the ontogeny development of *O.bimaculattus* larvae. It was observed that at 2 day post hatching (dph) mouth opened, teeth were visible and exogenous feeding started. The yolk sac disappeared within 3 dph. In course of the study, *O. bimaculatus* larvae were fed *Artemia* nauplii from 2 dph mixed zooplankton and chopped Tubifex during 4–7 dph, and only chopped Tubifex from 7 dph onwards.

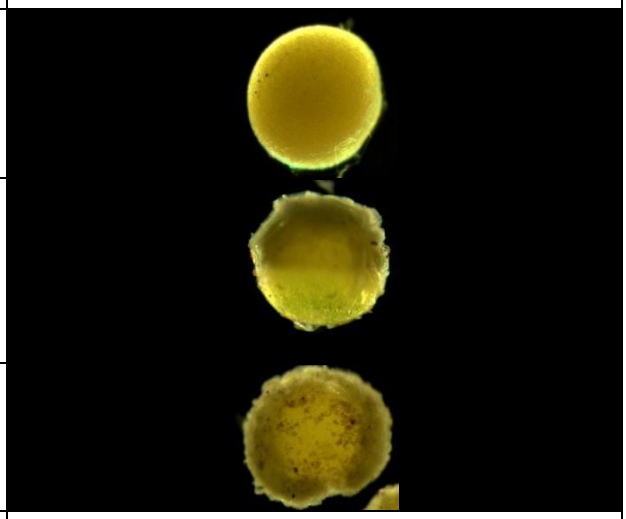
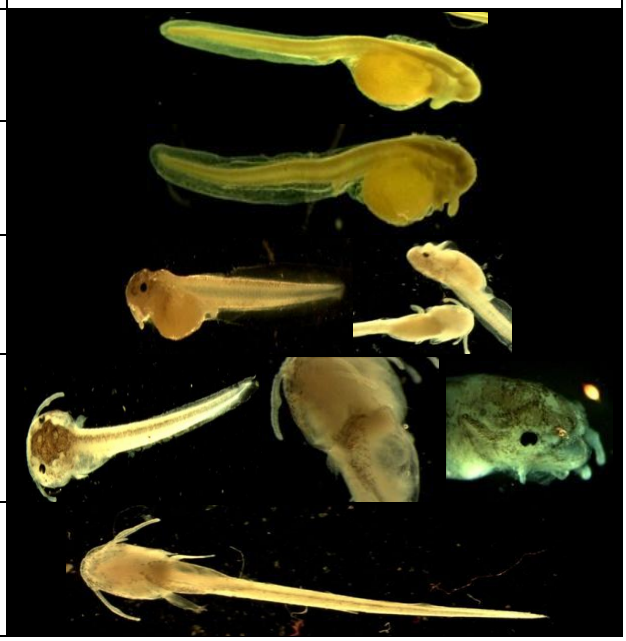
Catching of hatched out *O.bimaculatus* larvae

Collection of *O. bimaculatus* larvae

Collection of *O. bimaculatus* larvae

Collection of *O. bimaculatus* larvae

Table 3. Ontogeny: study of morphology

Time	Observation	Photograph(s)
3 hour post fertilization (HPF)	Gastrulation starts	
5:30 HPF	Dorsal (prospective head) and ventral (prospective tail) ends cover half of the egg; embryonic shield observed; ectoderm expands through epiboly	
8 HPF	Head and tail buds appear close together and cover entire yolk	
11 HPF	First movement starts near anal region; movement proceeds through tail region and tail detaches from yolk; eye spot becomes prominent.	
14 HPF	Embryo rotates circularly and outer layer of egg expands; anterior portion of head detaches from yolk	
15:30 HPF	Tail becomes visible outside the egg case	
17 HPF	Hatching starts and larva emerges from egg case; larval weight 0.5 mg, length 0.3 cm	
6 hour post hatching (HPH)	Anal opening becomes prominent and head becomes condensed	
1 day post hatching (DPH)	Larva with yolk sac and small barbells; weight 0.9 mg, length 0.5cm	
2 DPH	Yolk sac almost absorbed and mouth opens, teeth become visible; weight 1.20 mg, length 0.5-0.6 cm	
3 DPH	Weight 1.45 mg, length 0.8cm	

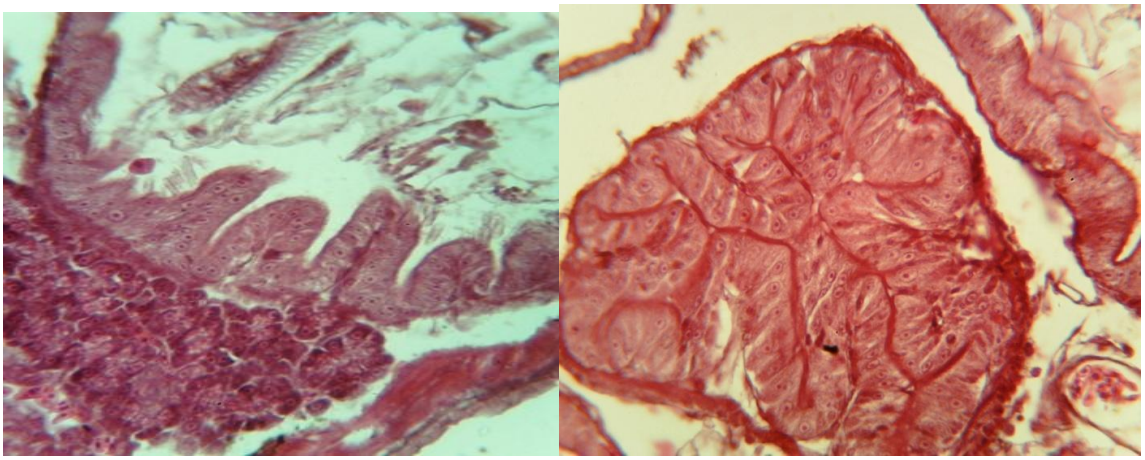
4 DPH	Fin rays become prominent; weight 3.4 mg, length 1 cm	
8 DPH	Intestine coiled and stomach divided; weight 62.6 mg, length 1.4 cm	
12 DPH	Weight 92.4 mg, length 1.7 cm, barbell 0.3 cm, gape of mouth 0.2 cm	
16 DPH	Weight 131.1 mg, length 2.4 cm, barbell 0.6 cm, gape of mouth 0.3 cm	
20 DPH	Weight 141.4 mg, length 3.2 cm, barbell 0.8 cm, gape of mouth 0.4 cm	
24 DPH	Weight 214.4 mg, length 4.5cm, barbell 1.0 cm, gape of mouth 0.6 cm	

Sample collection at Kalyani Field station

Sample preparation for enzyme analysis

Enzyme assay at the University of Burdwan

Figure 1. Ontogeny: histological study



A **B**

Fig: (A) Section of intestinal villi with food particles and (B) section of non glandular stomach at 7 day post hatching

Table 4. Digestive enzymes activities of *O. bimaculatus* from day 4 to day 24.

Day post hatching	Amylase	Total Protease	Trypsin	Chymotrypsin	Pepsin	Lipase
4	12.73±0.62	2.19±0.15	0.45±0.02	0.26±0.02	0.09±0.08	1.89±0.02
8	14.56±0.73	1.40±0.20	0.60±0.02	0.48±0.02	0.26±0.01	2.17±0.11
12	19.53±0.78	3.31±0.14	1.29±0.04	0.87±0.03	0.34±0.02	8.0±0.12
16	13.76±0.63	1.59±0.21	1.01±0.04	0.67±0.02	0.57±0.02	4.84±0.08
20	9.98±0.42	1.80±0.12	1.38±0.04	0.72±0.03	0.74±0.02	5.16±0.06
24	15.78±0.75	2.43±0.08	1.58±0.05	0.92±0.06	1.19±0.53	5.92±0.06

1. Trypsin and chymotrypsin activities (U) = 1µmol of 4-nitroaniline liberated min^{-1} mg^{-1} protein
2. Lipase activity (U) = 1µmol of free fatty acid liberated min^{-1} mg^{-1} protein
3. α-amylase activity (U) = µg maltose liberated min^{-1} mg^{-1} protein
4. Protease activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein
5. Pepsin activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein

Figure 2. Protein enzymes activity of *O. bimaculatus* larvae

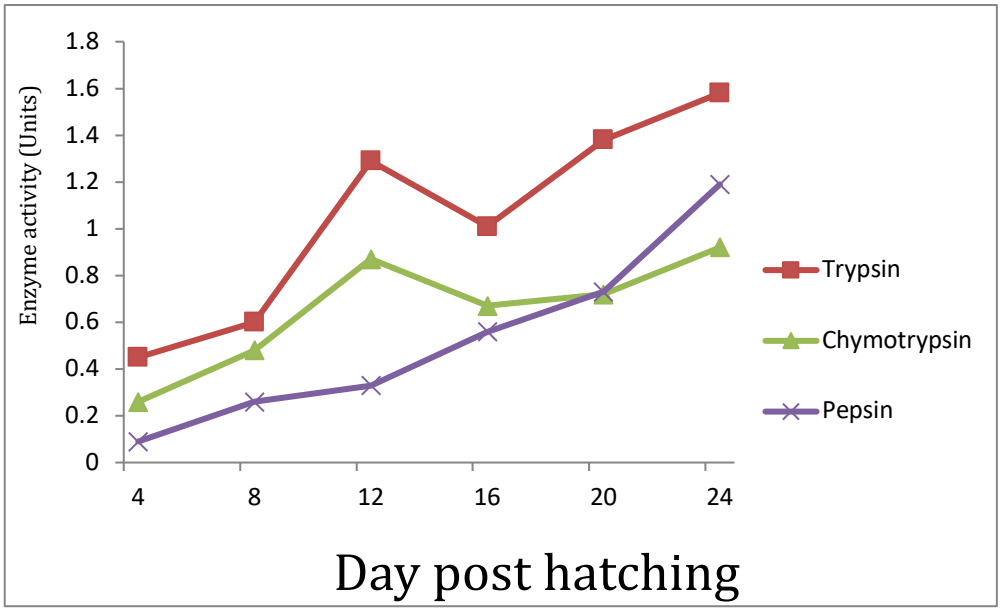


Figure 3. Digestive enzymes activity of *O. bimaculatus* larvae

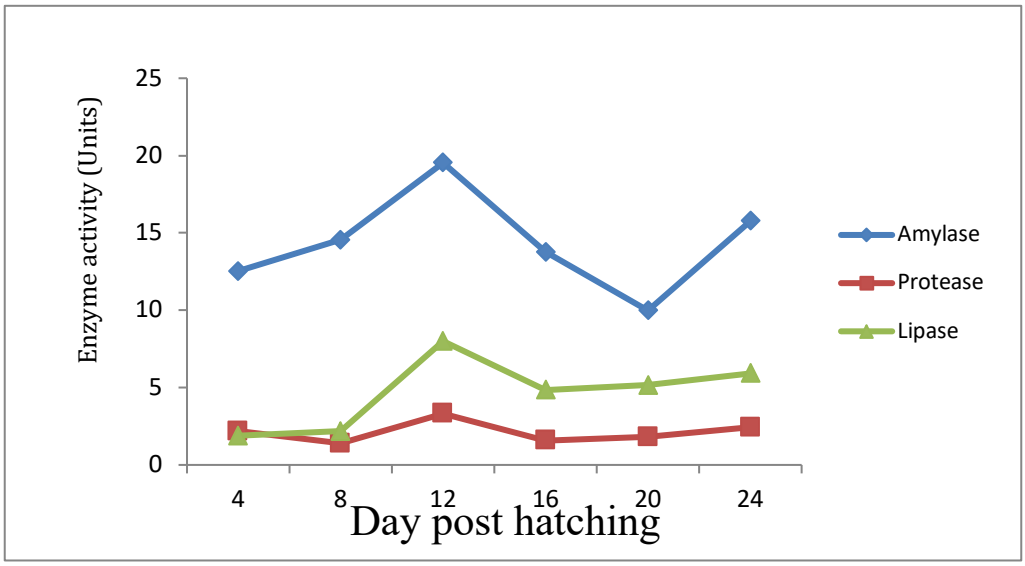


Figure 2 & 3 and table 4 describes the protein and digestives enzymes activity of *O. bimaculatus* larvae from day 4 to 24. Ther is gradual increase in the activity of the acid protease (pepsin) was noticed after formation of stomach, i.e. 8th day onward. Activities of amylase, lipase, trypsin and chymotrypsin were also detected in course of development post hatching

The activity of digestive enzymes indicated that enzymes involved in the digestion of proteins, lipids and carbohydrates were present in *O. bimaculatus* larvae since hatching (Figure 4 & 5), except pepsin. Coiling of intestine and presence of stomach were noticed by 8 dph, which was marked with the appearance of pepsin. Activities of the amylase, trypsin and chymotrypsin were increased initially after the onset of exotrophic phase, and decreased thereafter during 12 to 20 dph corresponding to the increase of pepsin. Thus, a change in the digestive physiology was indicated by progressive shift in the activity from alkaline (trypsin and chymotrypsin) to acid (pepsin) proteases.

Figure 4. Digestive Enzymes activities of *O. bimaculatus* larvae

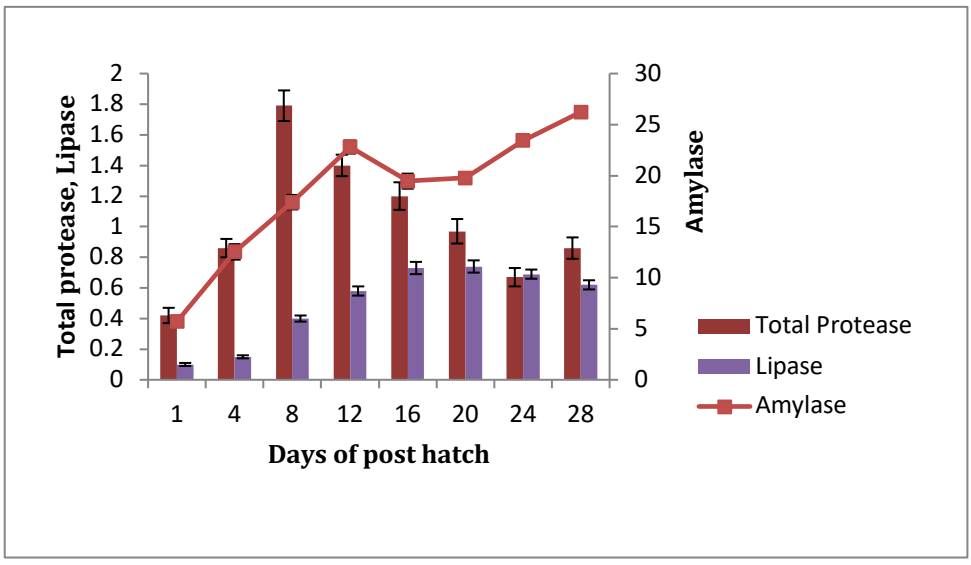


Figure 5. Protein enzymes activities of larvae

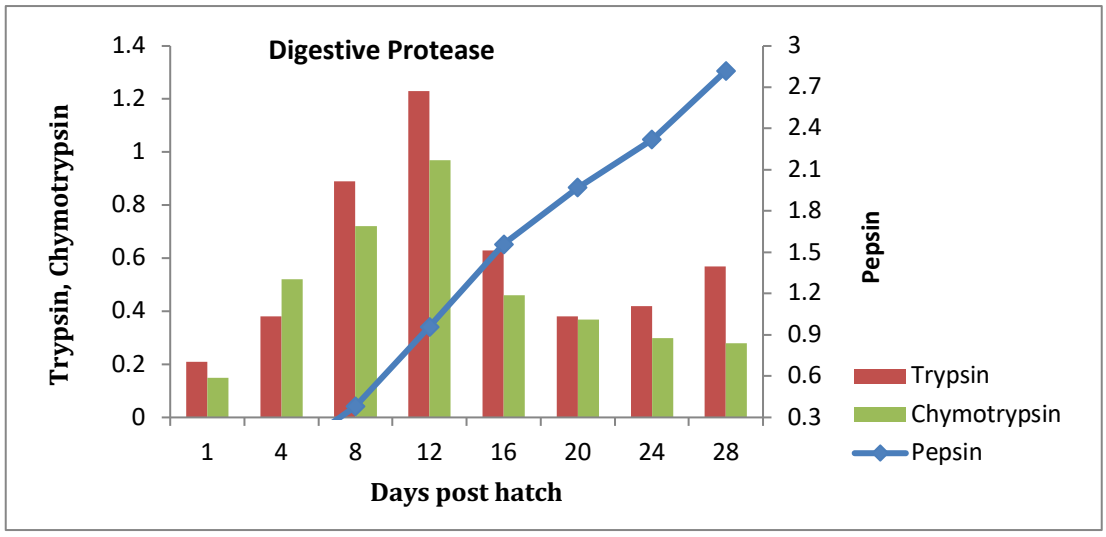


Figure 6. Carbohydrate metabolism enzymes of *O. bimaculatus* larvae

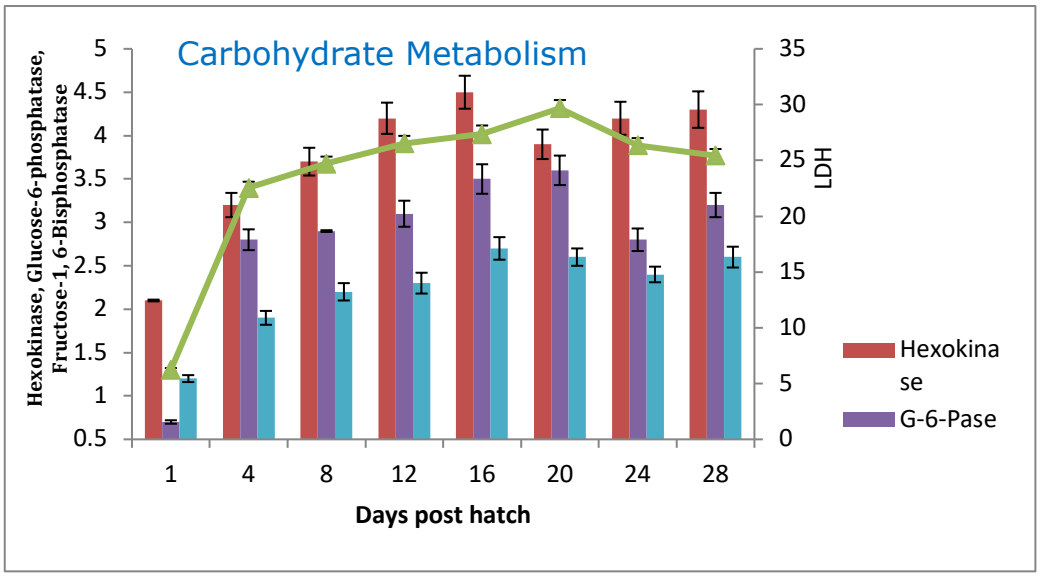


Figure 7. Amino Acid metabolism enzymes of larvae

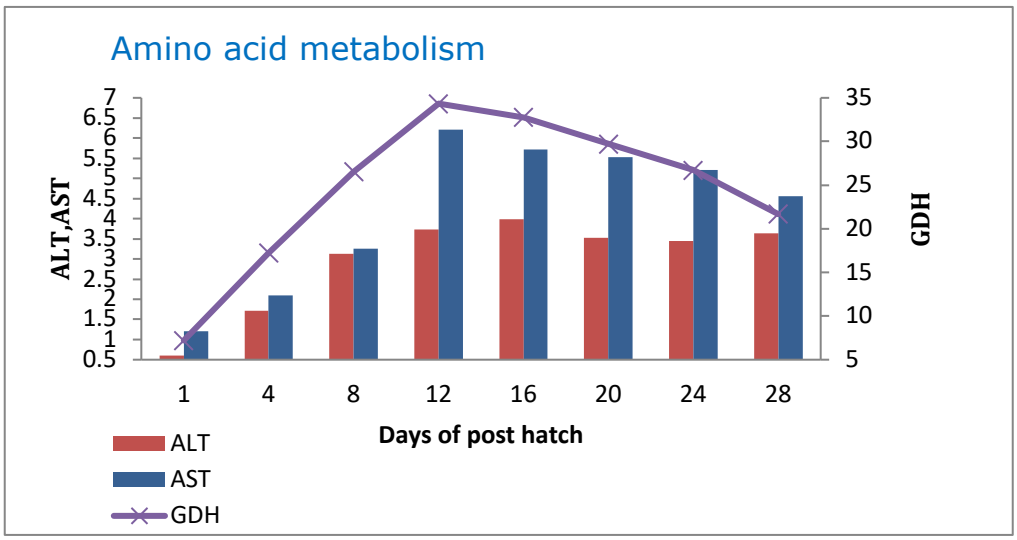


Figure 8. Carbohydrate Metabolic Enzymes of *O. bimaculatus* larvae

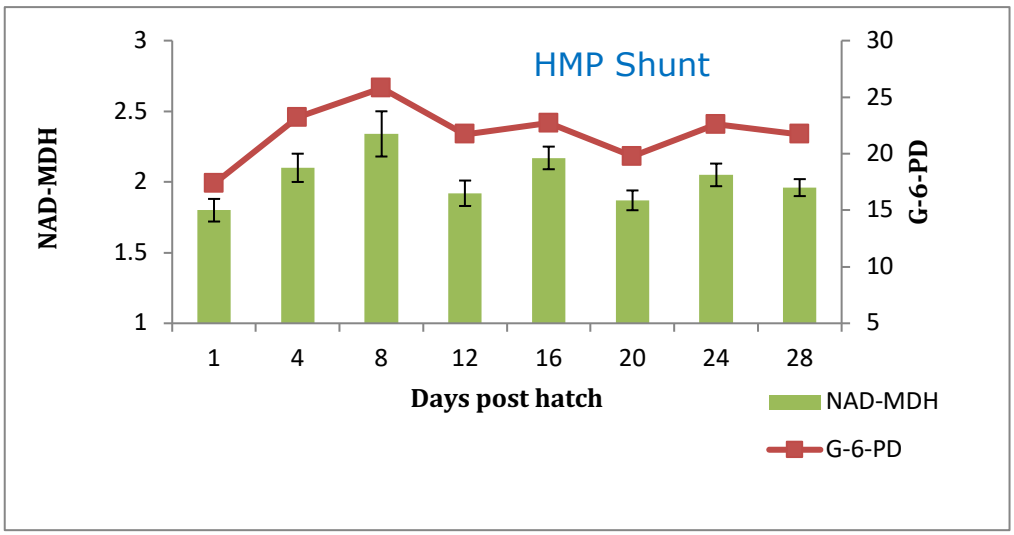


Figure 6, 7 and 8 shows the metabolic enzymes activities of *O. bimaculatus* larvae. The increase in Lactate dehydrogenase (LDH) activity from 4 to 20 dph was noticed, that could be indicative of stress. During this phase, increase in the activities of neoglucogenic enzymes (Glucose-6-phosphatase, Fructose-1, 6-bisphosphatase) was also noticed. However, a decreasing trend of LDH was apparent with increase in the Hexokinase

activity. Further, augmented Mitochondrial NAD-glutamate dehydrogenase activity coupled with increased Aspartate transaminase (and Alanine transaminase) at 12 dph was noticed signifying protein catabolism during this phase. Reduced neo glucogenic pathway at 4-8 dph was associated with high Glucose-6-phosphate dehydrogenase and cytosolic NADP-malate dehydrogenase activities that might indicate stress management and lipogenesis in this species during early development.

Activities of the enzymes indicated ontogenetic shift during the period of 8 to 20 dph, which could be associated with changes in food, gut morphology and shift in the activity pattern of digestive and metabolic enzymes. Overall, enzyme activities remained more or less stable beyond 20 dph.

Objective 3. To formulate larval feed and evaluation

Experiment No. 1: Feed evaluation with different levels of protein:

An experiment was conducted for 22 days initiated to study the protein requirement of *O. bimaculatus* larvae. The initial weight and length of *O. bimaculatus* larvae were 0.11±0.01 g and 20.49±0.70 mm. In the present experiment 3 formulated feeds were prepared with different graded levels of protein viz., Feed 1 (35%), Feed 2 (40%) and Feed 3 (45%). Fish meal, ground nut cake, soybean meal, wheat flour, vitamin and mineral mix and oil were used as feed ingredients in different proportions for preparation of experimental feed.

The feed formulations and proximate compositions of different feeds are presented in Table 5. The protein content of different feeds was 35.33±2.55, 40.69±0.55 and 45.23±0.17 respectively in Feed 1-3. Different proportions of fish meal, ground nut cake, soyabean meal, wheat flour etc were used to prepare the feed. Because of the graded increase in protein levels, the total ash contents of diets increased linearly from 15.40±0.39 to 16.37±0.39, which could be attributed to the presence of higher levels of fish meal as a major feed ingredient.

Water quality parameters were recorded as 28-30^{0}C, 7.4-7.8, 5.0-5.8 mg/l and 235-240mg/l $CaCO_3$ for temperature, pH, dissolved oxygen and total alkalinity respectively. Dissolved oxygen play a vital role in rearing of larvae because larvae require optimum level of oxygen for sustaining their physiological condition

The growth performance of *Ompok* larvae feed with different levels of Protein (Table no 6). Initially the larvae were fed with natural food and their formulated feed. The initial body weight, net weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and survivability are shown in Table 6. The initial weight of larvae were 0.11±0.004, 0.106±0.004 and 0.106±0.004 (g) in different feed Treatments (Feed 1-3) respectively while the average final weights after 22 days of experimental period was 0.34±0.06, 0.91±0.15 and 0.36±0.07 (g) in the respective treatments. The net weight gain were 0.23±0.05, 0.81±0.15 and 0.26±0.06 (g)respectively in Feed 1-3, which showed that the highest gain in weight of the larvae was 0.81±0.15 (g) in treatment Feed 2 followed by Feed 1 and Feed 3 . The net weight gain was significantly ($P<0.01$) higher in Feed 2 (Table 6 and Fig 9). The survival (%) was 73.0±2.02, 71.0±1.0 and 59.0±4.7 respectively in Feed 1-3. Significantly higher specific growth rate (SGR) was observed in Feed 2 and followed by Feed 1 and Feed 3. The final weight, net weight gain and

specific growth rate was significantly (P<0.05) higher in Feed 2 compared to those of Feed 1 and Feed 3. The value of feed conversion ratio (FCR) was significantly (P>0.05) lower in Feed 2 while protein efficiency ratio (PER) was non-significant among all the Feed treatments. Lowest FCR in Feed 2 indicated that lower amount of feed needed to produce one unit of fish biomass. So lower the FCR is, better the efficiency of the feed is and such type of feed in the present experiment was Feed 2.

Table 5. Ingredients and proximate composition (%DM basis) of experimental diets

Particulars	Feed 1	Feed 2	Feed 3
Fish Meal	44	53	65
Ground nut cake	15	15	15
Soyabean Meal	10	10	5
Wheat flour	19	10	03
CMC	2	2	2
Vit. & min. Mixture	5	5	5
Veg oil	5	5	5
Proximate Composition (% DM basis)			
Dry Matter	93.02±0.93	92.39±0.22	92.34±1.33
Crude Protein	35.33±2.25	40.69±0.55	45.23±0.17
Crude lipid	9.11±0.47	9.49±0.31	9.61±0.31
Total ash	15.40±0.39	16.35±0.09	16.37±0.39

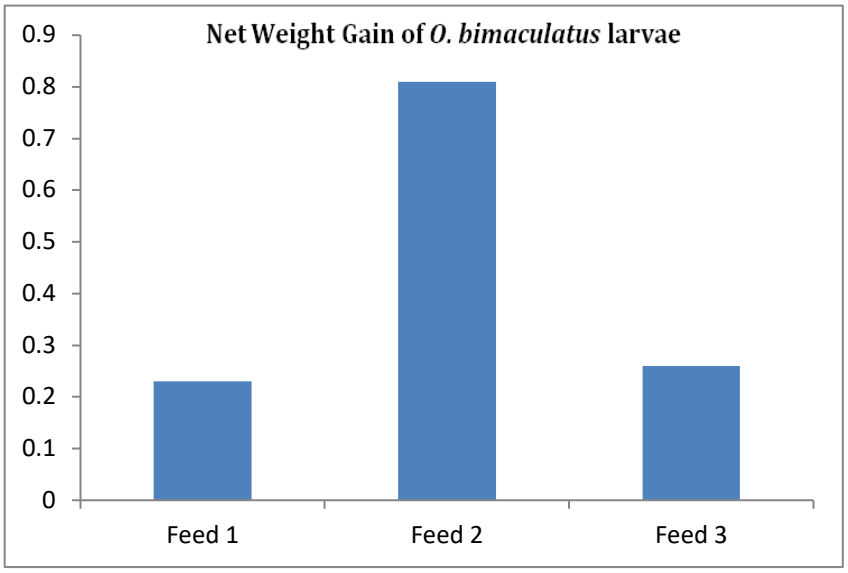
Data are presented as Mean± S.E. CMC- Carboxy methyl cellulose.

Table 6. Growth of *O.bimaulatus* larvae fed with different levels of Protein.

Particulars	Feed 1	Feed 2	Feed 3
Initial weight(g)	0.11±0.004	0.106 ±0.008	0.106±0.008
Final weight(g)	0.34 ± 0.06^{a}	0.91 ± 0.15^{b}	0.36 ± 0.07^{ab}
NWG(g/22d)	0.23 ± 0.05^{a}	0.81 ± 0.15^{b}	0.26 ± 0.06^{a}
SGR(%/d)	4.54 ± 0.60^{a}	7.97 ± 0.76^{b}	5.30 ± 0.53^{a}
FCR	3.62 ± 0.51^{b}	1.93 ± 0.43^{a}	3.68 ± 0.35^{b}
PER	0.82±0.13	1.42±0.33	0.62±0.05
Survivability	73.5±2.02	71.0±1.0	59.0±4.7
DNA(µg/mg)	30.59±0.30	34.31±0.43	31.04±0.54
RNA(µg/mg)	34.86±0.41	45.16±0.36	35.65±0.31
RNA/DNA	1.14±0.07	1.32±0.04	1.15±0.04

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly ($P<0.01$)

Figure. 9. Net weight gain of *O.bimaculatus* fed with different levels of Protein



Sampling of *O.bimaculatus* larvae

Weight measurement

Length measurement

Table 7. Enzyme contents of *O. bimaculatus* larvae fed with different levels of Protein

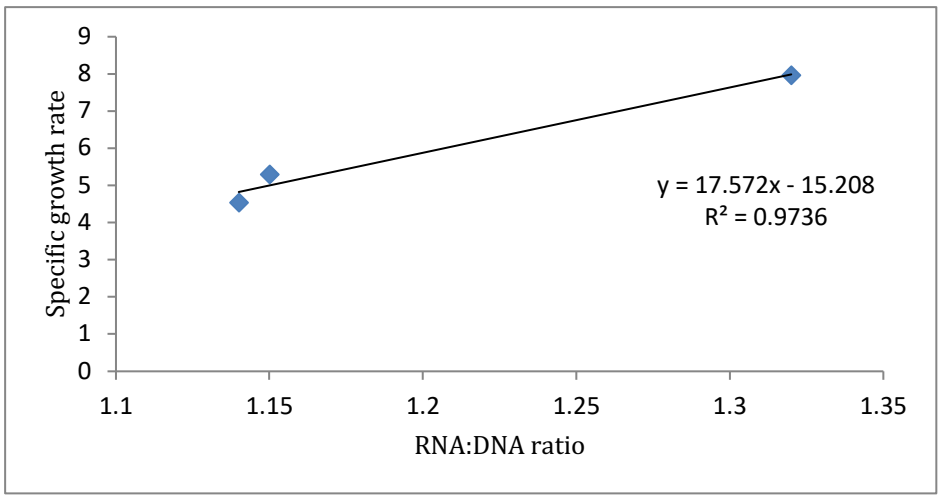
Particulars	Initial	Feed 1	Feed 2	Feed 3
Amylase*	17.4±0.33^{a}	23.87±0.17^{b}	25.36±0.34^{c}	25.46±0.47^{c}
Total Protease$^{¥}$	1.76±0.03^{d}	0.67±0.02^{a}	0.81±0.01^{b}	0.73±0.01^{c}
Lipase$^{\#}$	0.4±0.01^{a}	0.57±0.02^{b}	0.69±0.03^{d}	0.61±0.02^{c}
Pepsin$^{¥}$	0.38±0.01^{a}	2.53±0.02^{b}	2.67±0.01^{c}	2.49±0.01^{b}
Trypsin¶	0.89±0.02^{d}	0.20±0.01^{a}	0.18±0.02^{a}	0.26±0.03^{c}
Chymotrypsin¶	0.72±0.02^{c}	0.29±0.02^{b}	0.19±0.01^{a}	0.28±0.03^{b}
ALT(Alanine transaminase) $^{€}$	3.14±0.03^{a}	3.44±0.02^{b}	3.51±0.02^{c}	4.25±0.02^{d}
AST(Aspartate transaminase) ^{ƨ}	3.24±0.02^{a}	4.61±0.03^{c}	4.38±0.01^{b}	5.12±0.02^{d}

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly (P<0.05)

* mg maltose liberated mg^{-1} protein h$^{-1,}$ $^{¥}$µg of tyrosine liberated mg^{-1} protein min^{-1}, $^{\#}$ µM of fatty acid liberated mg^{-1} protein min^{-1}, ¶1µmol of 4-nitroaniline liberated min^{-1} mg^{-1} protein Enzyme activity units mg^{-1} protein min^{-1}, $^{€}$ µM of pyruvate formed mg^{-1} protein min^{-1}, ^{ƨ} µM of oxaloacetate formed mg^{-1} protein min^{-1}

Perusal of table 7 reveals that Amylase activity of feed 1 was significantly differed from feed 2 and feed 3 diet. Lipase and pepsin activity was significantly (P<0.05) higher in feed 2. Trypsin and Chymotrypsin activity significantly (P>0.05) lower in feed 2. ALT and AST activities changes were significantly (P<0.05) higher in Feed 3 but both AST and ALT activities were significantly (P>0.05) lower in feed 2.

Figure 10. Relation between SGR and RNA-DNA ratio in *O. bimaculatus* Larvae



The DNA, RNA and RNA/DNA ratio of larvae was non significant among the fed treatments. . The Relationship between SGR and RNA/DNA ratio in *O. bimaculatus* has depicted that RNA/DNA ratio was highly correlated with Feed 2 having 40% protein vis-à-vis other Groups(Figure 10). The present experiment revealed that 40% crude protein was sufficient for the optimum growth and survival of *O. bimaculatus* larvae and it also revealed that the amylase, lipase and pepsin enzymes was significantly ($P<0.05$) higher in Feed 2 having 40% protein.

Experiment No. 3: Feed evaluation with Lipid requirement for *Ompok bimaculatus* larvae

An experiment was conducted 42 days to study the lipid requirement of *O. bimaculatus* larvae. The initial weight and length of *O. bimaculatus* larvae were 0.15±0.003 g and 22.65±1.70 mm. In the present experiment 3 formulated feeds were prepared having different lipid levels viz., Feed 1 (4.5%), Feed 2 (7.0%) and Feed 3 (9.5%).

The Proximate composition and feed formulation is presented in Table 8. The feed ingredients viz., fish meal, ground nut cake, soyabean meal, wheat flour, fish and vegetable oil (1:1) and vitamin and mineral mixture were used in different proportions for preparation of feed. Perusal of table 8 reflects that the protein content of different feeds were 40.46±0.06, 40.18±0.49 and 40.61±0.83 (%) respectively in Feeds1-3. The analysed lipid content of different feeds were 5.7±0.2, 8.0±0.25 and 10.45±0.45 respectively in Feed treatments 1-3.

Experimental work at wet Laboratory of RRC, Rahara

Table 8: Feed formulation and proximate composition (%DM Basis)

Particulars	Feed 1	Feed 2	Feed 3
FM	53	53	53
GNOC	15	15	15
SBM	10	10	10
Wheat flour	10.50	8.0	5.50
CMC	2.0	2.0	2.0
Fish :Veg oil (1:1)	4.5	7.0	9.5
Vit-Min mix	5	5	5
Proximate composition (%DM Basis)			
Dry matter	92.85±0.06	92.37±0.23	92.06±0.05
Crude protein	40.46±0.06	40.18±0.49	40.61±0.83
Crude lipid	5.7±0.2	8.0±0.25	10.45±0.45
Total Ash	14.4±0.3	15.4±0.2	16.5±0.3

Fig. 11: Net weight gain of *O. bimaculatus* larvae fed with different lipid levels

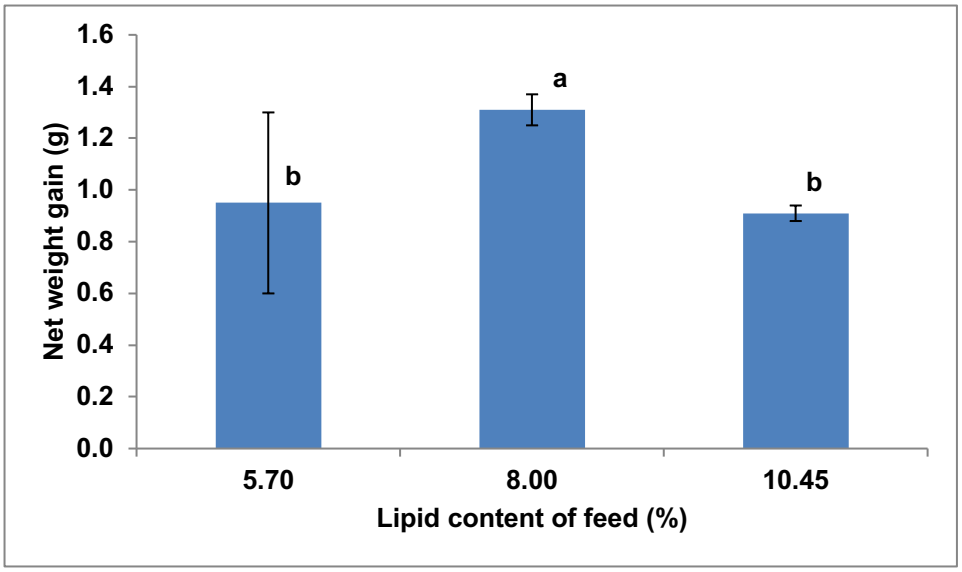


Table 9: Growth Performance of *O. bimaculatus* larvae fed with different level of lipids

Particulars	Feed 1	Feed 2	Feed 3
Initial Weight (g)	0.15±0.003	0.14±0.01	0.15±0.01
Final Weight (g)	1.10±0.12^{a}	1.40±0.07^{b}	1.06±0.03^{a}
Net weight gain (g/42d)	0.95±0.12^{a}	1.31±0.06^{b}	0.91±0.03^{a}
Specific Growth Rate (%/day)	4.73±0.35^{a}	5.50±0.05^{b}	4.66±0.22^{a}
DGC	0.73±0.02^{a}	1.003±0.05^{b}	0.76±0.09^{a}
Survivability (%)	83.85±6.15	83.85±6.15	79.55±5.46
FCR	1.86±0.10^{b}	1.39±0.05^{a}	1.74±0.07^{b}
PER	1.31±0.09^{a}	2.39±0.17^{b}	1.30±0.08^{a}

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly (P<0.01)

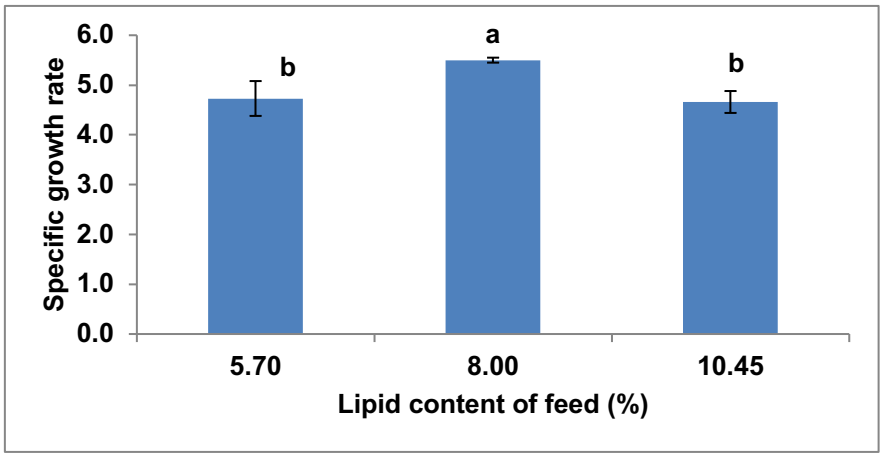
Sampling of *O. bimaculatus* larvae

The water quality parameters were: temp 28-30^{0}C, pH 6.8-7.7, dissolved oxygen 6.8-74 mg/l and total alkalinity 230-240 mg/l. Perusal of Table 9 shows the growth performance of *O. bimaculatus* larvae reared with different lipid levels. The final weight of fish were 1.10±0.12, 1.40±0.07 and 1.06±0.03 g respectively in Feed 1-3. The final weight was significantly (P<0.01) higher in Feed 2 having 8 % lipid. The net weight gain were 0.95±0.12, 1.31±0.06 and 0.91±0.03 (g) respectively in feed treatments Feed 1-3 (Figure 11). The specific growth rate (SGR) were 4.73±0.35, 5.50±0.05 and 4.66±0.22 respectively in Feed 1-3. The net weight gain, DGC and specific growth rate was significantly (P<0.05) higher in Feed 2 having 8% lipid. The Survivability

(%) were 83.85±6.15, 83.85±6.15 and 79.55±5.46 respectively in Feeds 1-3. The feed conversion ratio (FCR) were1.86±0.10, 1.39±0.05 and 1.74±0.07 in Feed treatments 1-3. He FCR was significantly (P< 0.05) lower in Feed 2 group.

***O.bimaculatus* larvae after 42 days of experiment**

Fig. 12: Specific Growth rate of *O. bimaculatus* larvae reared with different lipid levels



The protein efficiency ratio (PER) were 1.31±0.09, 2.39±0.17 and 1.30±0.08 respectively in Feed 1-3. The protein efficiency ratio was significantly (P<0.05) higher in Feed 2 (Figure 12).

Recording of length of pabda larvae Recording of weight of pabda larvae

Table 10 Carcass composition (% as such) of *O.bimaculatus* larvae fed different level of lipid

Particulars	Feed 1	Feed 2	Feed 3
Moisture	79.37±0.09^{a}	80.93±0.22^{b}	79.80±0.17^{a}
Crude Protein	13.93±0.09	14.40±0.21	14.03±0.08
Fat	2.50±0.06^{a}	2.90±0.12^{b}	2.77±0.0^{b}
Ash	1.70±0.06	1.97±0.09	1.80±0.06

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly (P<0.01)

The carcass composition of *O. bimaculatus* larvae are presented in table 10. Perusal of table 10 reflects that the moisture and fat content carcass tissue of pabda larvae differed significantly (P<0.05) among the feed treatments. The moisture and fat content was significantly higher in Feed 2, and it did not differ significantly with Feed 3. Protein and ash content did not differ significantly among the feed treatments.

Table 11 Digestive enzyme activity of *O.bimaculatus* larvae

Particulars	Feed 1	Feed 2	Feed 3
Amylase$^{\alpha}$	4.42^{b}±0.21	4.02^{b}±0.19	2.92^{a}±0.13
Protease$^{\beta}$	1.42^{b}±0.06	1.54^{b}±0.07	1.26^{a}±0.04
Lipase$^{\pi}$	1.34^{b}±0.05	1.42^{b}±0.06	1.19^{a}±0.07

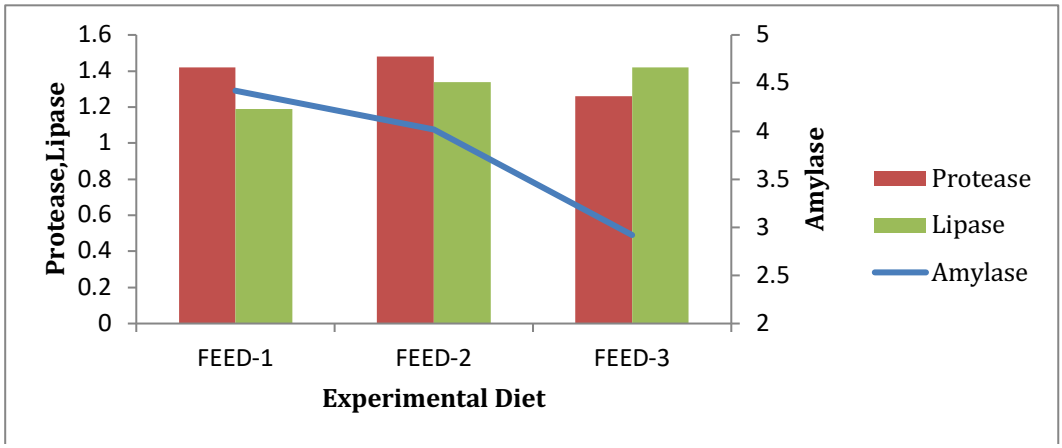
Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly (P<0.05)

$^{\alpha}$Amylase activity (U) = µg maltose liberated min^{-1} mg^{-1} protein

$^{\beta}$Protease activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein

$^{\pi}$Lipase activity (U) = 1µmol of free fatty acid liberated min^{-1} mg^{-1} protein

Fig. 13: Digestive enzyme activities of *O. bimaculatus* larvae fed different levels of lipids



The activities of digestive enzymes in *O. bimaculatus* were assayed by feeding different levels of lipids as given in Table 11. The activities of digestive enzymes in pabda larvae revealed that amylase, Protease and lipase activity was significantly ($P<0.05$) higher in F_2 and it did not differ significantly with F_1.

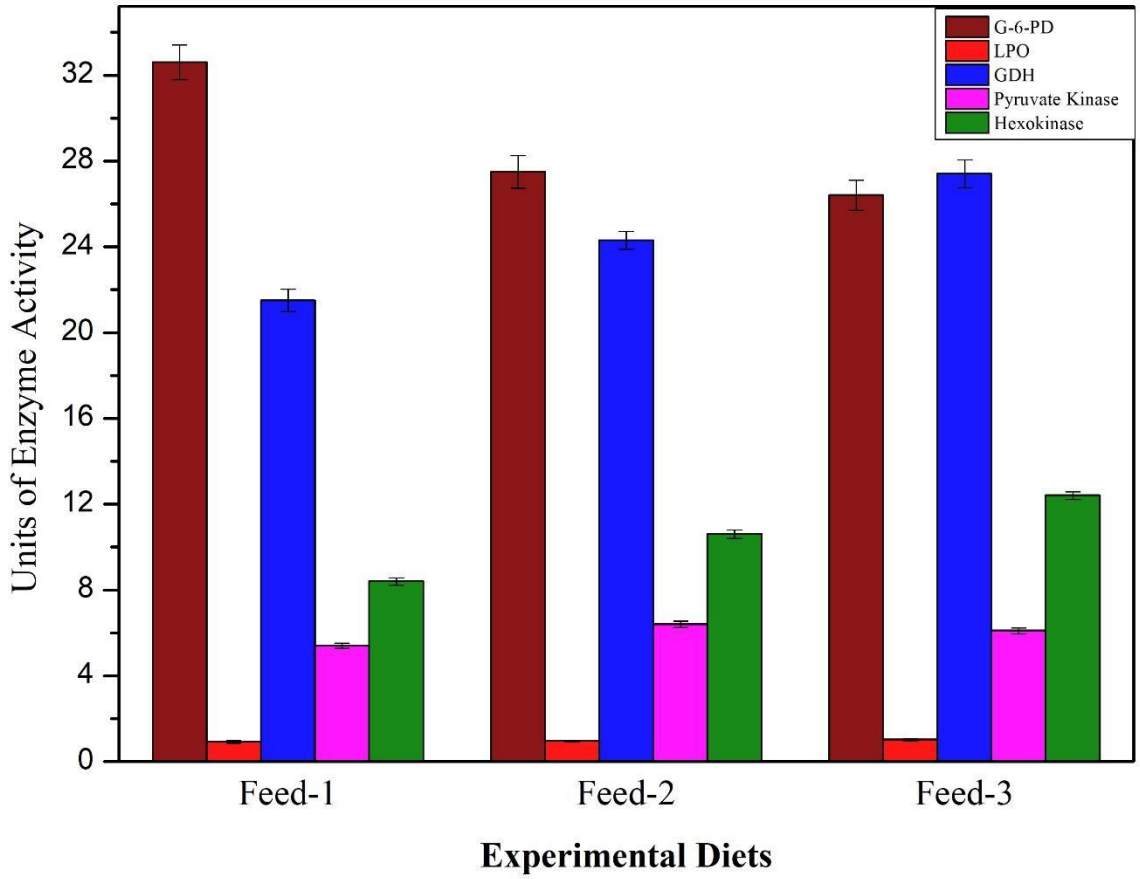
Table 12 Metabolic enzymes of *O.bimaculatus* larvae fed with different levels of lipid

Particulars	Feed 1	Feed 2	Feed 3
Glucose 6 Phosphate Dehydrogenase$^{\alpha}$	$32.6^{a}\pm0.81$	$27.5^{b}\pm0.76$	$26.4^{b}\pm0.7$
Glutamate Dehydrogenase$^{\beta}$	$21.5^{a}\pm0.53$	$24.3^{b}\pm0.42$	$27.4^{c}\pm0.65$
Pyruvate kinase$^{\mu}$	$5.4^{a}\pm0.14$	$6.4^{b}\pm0.14$	$6.1^{b}\pm0.14$
Hexokinase$^{\epsilon}$	$8.4^{a}\pm0.17$	$10.6^{b}\pm0.19$	$12.4^{c}\pm0.17$
Lipid Peroxidation$^{\phi}$	0.92±0.06	0.96±0.04	1.02±0.06

Data are presented as Mean± S.E. ^{a,b} Means with different superscripts in a row differ significantly ($P<0.05$)

$^{\alpha}$G-6-PD(U)& NAD-MDH(U) = µM of NADPH formed mg protein^{-1} h^{-1}
$^{\beta}$GDH(U)= µM of Fomazan formed mg protein^{-1} h^{-1}
$^{\mu}$Pyruvate kinase^{+}µM of NAD formed mg protein^{-1} h^{-1}
$^{\epsilon}$Hexokinase(U)= µM of NAD formed mg protein^{-1} h^{-1}
$^{\phi}$Lipid Peroxidation (U)= µM thiobarbituric acid reactive substance (TBARS) formed mg protein^{-1} min^{-1}

Figure 14. Metabolic enzymes of *O.bimaculatus* larvae fed with different levels of lipid



The metabolic enzymes activity is presented on table 12. Glutamate dehydrogenase and Hexokinase enzyme was significantly ($P<0.05$) higher in larvae fed feed-3. Glucose 6 phosphatase dehydrogeanse and pyruvate kinase was significantly ($P<0.05$) higher in F_2 and it did not differ significantly with F_3. He lipid peroxidation enzyme did not differ significantly among the feed treatments. The present experiment reveals that 8% lipid is sufficient for the optimum growth and survival of *O. bimaculatus* larvae. The present experiment reveals that 8% lipid is sufficient for the optimum growth and survival of *O. bimaculatus* larvae.

Experiment no. 4: Feed evaluation with different feed additives for *O. bimaculatus*

The study aimed at evaluation of animal protein (on wet basis) supplemented to dry formulated feed of the butter catfish, *Ompok bimaculatus* (Bloch) juveniles. Hatchlings were produced by induced breeding of mature *O. bimaculatus* using a hormone analogue (synthetic GnRH) at the Department of Zoology, University of Burdwan, Golapbagh, Burdwan, West Bengal. Juveniles were raised in the laboratory and 30 day old juveniles (0.44±0.02 g) were stocked in five groups in triplicate for 50 days. A formulated basal diet (40% crude protein) was fed to the control group (D1), whereas diets supplemented with (10%, wet weight) blood meals (D2), chicken viscera (D3), egg albumin (D4) and mussel meat (D5) were fed to the experimental groups for 90 days.

Five experimental diets were prepared along with Fish meal, Soya bean meal, Ground nut cake, wheat flour, veg. oil & Vitamin and mineral mixture with 40 % protein. Feeds were prepared, sterilized and stored at -20^0 C and fresh protein sources viz., blood meal,chicken viscera, egg albumin and mussel meat were mixed daily before feeding. The experimental fish were fed twice daily at 09:00h and 15:00h at a fixed feeding rate. Water quality was maintained in all all experimental tanks by daily renewal of 30% of their total volume.

The feed ingredient composition and proximate composition is presented in Table 13. In diet D-2, D-3, D-4 and D-5; animal protein component (10% w/w) viz., Blood Meal (D-2), Chicken Viscera (D-3), Egg albumin (D-4) and Mussel meat (D-5) were supplemented in the control diet. The protein content of different feeds were 40.14±0.55, 40.89±0.41, 41.64±0.67, 41.24±0.54 and 41.56±0.72 (%) respectively in diets D-1 to D-5.

The growth performance and feed utilization efficiencies of *O. bimaculatus* juveniles fed with different animal protein supplements is presented in Table 14 and Figure 15. The final weight were 4.81±0.07, 4.65±0.06, 5.36±0.05, 5.85±0.06 and 5.46±0.04 (g) respectively in diets D-1 to D-5.

Table 13. Ingredient and Proximate composition (% DM) of different Experimental diets

Particulars	D-1	D-2	D-3	D-4	D-5
Fish Meal	53	53	53	53	53
Soybean Meal	15	15	15	15	15
Groundnut cake	10	10	10	10	10
Wheat Flour	10	10	10	10	10
Sunflower oil	5	5	5	5	5
Agrimin (Vit. & Min. Mix.)	5	5	5	5	5
Carboxy methyl cellulose	2	2	2	2	2
Animal protein supplement (10% w/w)	-	Blood Meal	Chicken Viscera	Egg albumin	Mussel Meat
Proximate composition					
Dry Matter	92.39±0.32	92.86±0.41	93.94±0.56	92.95±0.53	93.62±0.74
Crude Protein	40.14±0.55	40.89±0.41	41.64±0.67	41.24±0.54	41.56±0.72
Crude Lipid	9.48±0.31	9.67±0.24	9.64±0.35	9.51±0.47	9.78±0.36
Ash	16.35±0.09	16.45±0.12	16.76±0.04	16.48±0.07	16.53±0.36

The final weight was significantly ($P<0.05$) higher in Diet-4 vis-à-vis other diets. The FCR and SGR were 1.63±0.08, 1.69±0.10, 1.51±0.07, 1.32±0.06 and 1.38±0.06; 2.65±0.06, 2.62±0.05, 2.80±0.4, 2.87±0.07 and 2.78±0.05 respectively in Diets D-1 to D-5. The FCR was significantly ($P<0.05$) lower in D-4 and SGR was significantly higher in D-4.. The ANPU were 24.20±0.45, 22.43±0.35, 26.53±0.52, 33.76±0.47 and 28.28±0.6 respectively in D-1 to D-5. The ANPU was significantly ($P<0.05$) higher in D-4 group.

The diet D4 exhibited better performance in terms of growth, feed conversion ratio, specific growth rate, protein efficiency ratio and apparent net protein utilization. The mortality(%) was significantly lower in D-4 and D-2.

Table14. Growth performances and feed utilization efficiencies in *Ompok bimaculatus*

Particulars	D-1	D-2	D-3	D-4	D-5
Initial weight(g)	0.44±0.20				
Final weight (g)	4.81±0.07^{a}	4.65±0.06^{b}	5.36±0.05^{c}	5.85±0.06^{d}	5.46±0.04^{c}
FCR	1.63±0.08^{b}	1.69±0.10^{b}	1.51±0.07^{a}	1.32±0.06^{a}	1.38±0.06^{a}
SGR	2.65±0.06^{a}	2.62±0.05^{a}	2.80±0.4^{b}	2.87±0.07^{b}	2.78±0.05^{b}
Mortality (%)	17.14±0.71^{b}	14.29±0.10^{a}	18.21±0.71^{b}	14.29±0.12^{a}	21.42±0.95^{c}
ANPU	24.20±0.45^{a}	22.43±0.35^{a}	26.53±0.52^{b}	33.76±0.47^{d}	28.28±0.61^{c}
PER	1.52±0.03^{b}	1.37±0.03^{a}	1.53±0.04^{b}	1.84±0.04^{d}	1.68±0.04^{c}

Data are Means ± Standard error (n=3). Means with different superscript in a row are significant ($P < 0.05$)

The PER were 1.52±0.03, 1.37±0.03, 1.53±0.04, 1.84±0.04 and 1.68±0.04 respectively in diets D-1 to D-5. The PER was significantly ($P<0.05$) higher in D-4 group.

Table 15. Carcass composition of *O.bimaculatus* juveniles feed with different Protein supplements (w/w)

Particulars	D-1	D-2	D-3	D-4	D-5
Moisture	77.37±0.57^{a}	77.17±0.54^{a}	77.99±0.59^{a}	78.23±0.52^{b}	77.93±0.53^{a}
Protein	14.84 ±0.22^{a}	14.98±0.24 ^{a}	15.49±0.26^{b}	16.19±0.27^{c}	15.79±0.28^{b}
Lipid	2.47±0.12 ^{a}	2.46±0.90 ^{a}	2.55±0.14^{b}	2.51±0.10 ^{a}	2.88±0.13^{b}

Data are Mean ± S.E. Means with different superscript in a row are significant ($P < 0.05$)

The carcass composition of juvenile *O. bimaculatus* are presented in Table 15. The moisture and protein content were significantly ($P<0.05$) higher in D-4. However, the lipid content was significantly ($P<0.05$) higher in D-3and D-5. The experimental diet D4 exhibited significantly ($P<0.05$) higher growth, feed conversion ratio, specific growth rate and carcass protein deposition.

Table 16. Activities of enzymes of *Ompok bimaculatus* fed with different feed additives

Enzymes	D-1	D-2	D-3	D-4	D-5
Amylase	11.40 ± 0.52^{a}	16.59 ± 0.62^{b}	17.60 ± 0.54^{b}	16.22 ± 0.53^{b}	12.77 ± 0.54^{a}
Protease	1.13 ± 0.07^{c}	1.32 ± 0.04^{c}	0.79 ± 0.05^{b}	0.55 ± 0.04^{a}	0.54 ± 0.06^{a}
Lipase	1.82 ± 0.04^{a}	1.70 ± 0.04^{a}	2.24 ± 0.05^{c}	2.35 ± 0.06^{c}	2.01 ± 0.04^{b}
Pepsin	3.25 ± 0.06^{a}	3.35 ± 0.07^{a}	3.89 ± 0.09^{b}	4.35 ± 0.12^{c}	4.0 ± 0.10^{b}
Trypsin	0.57 ± 0.04^{c}	0.56 ± 0.06^{c}	0.43 ± 0.05^{b}	0.37 ± 0.05^{a}	0.36 ± 0.04^{a}
Chymotrypsin	0.43 ± 0.05^{b}	0.54 ± 0.06^{b}	0.33 ± 0.04^{a}	0.27 ± 0.05^{a}	0.23 ± 0.05^{a}
Alanine transaminase (ALT)	1.65 ± 0.09^{c}	1.75 ± 0.12^{c}	1.62 ± 0.08^{b}	1.36 ± 0.08^{a}	1.67 ± 0.09^{b}
Asparate transaminase (AST)	5.77 ± 0.12^{b}	6.03 ± 0.14^{c}	5.73 ± 0.10^{b}	5.49 ± 0.13^{a}	5.78 ± 0.08^{b}

Data are Mean ± S.E. Means with different superscript in a row are significant ($P < 0.05$)

Trypsin and chymotrypsin activities (U) = 1µmol of 4-nitroaniline liberated min^{-1} mg^{-1} protein

Lipase activity (U) = µmol of free fatty acid liberated min^{-1} mg^{-1} protein

α-amylase activity (U) = µg maltose liberated min^{-1} mg^{-1} protein

Protease activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein

Pepsin activity (U) = µg tyrosine liberated min^{-1} mg^{-1} protein

ALT & AST (U) = nmol sodium pyruvate released min^{-1} mg^{-1} protein

The enzymes activities of *Ompok bimaculatus* fed with different feed additives are presented in Table 16 and figure 16 & 17. Activities of amylase and lipase were significantly higher in groups D3 and D4, respectively, as compared to the other groups. Diet D4 sustained the highest pepsin activity (4.35±0.12 U); however, activities of the alkaline proteases (Trypsin, Chymotrypsin) were reduced in fish fed diets D4 and D5. Significantly lower aspartare aminotransferase (AST) and alanine amniotransferase (ALT) activities in the liver were recorded in *O.bimaculatu*s juveniles fed Diets D4 and D5, respectively.

Figure15.GrowthPerformanceof*O.bimaculatus*

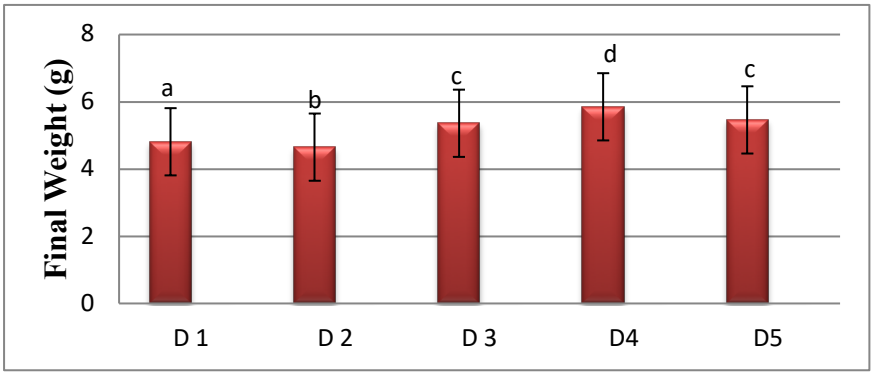


Fig 16. Activities of Protease, Lipase and Amylase in *O. bimaculatus* fed with different Feed additives

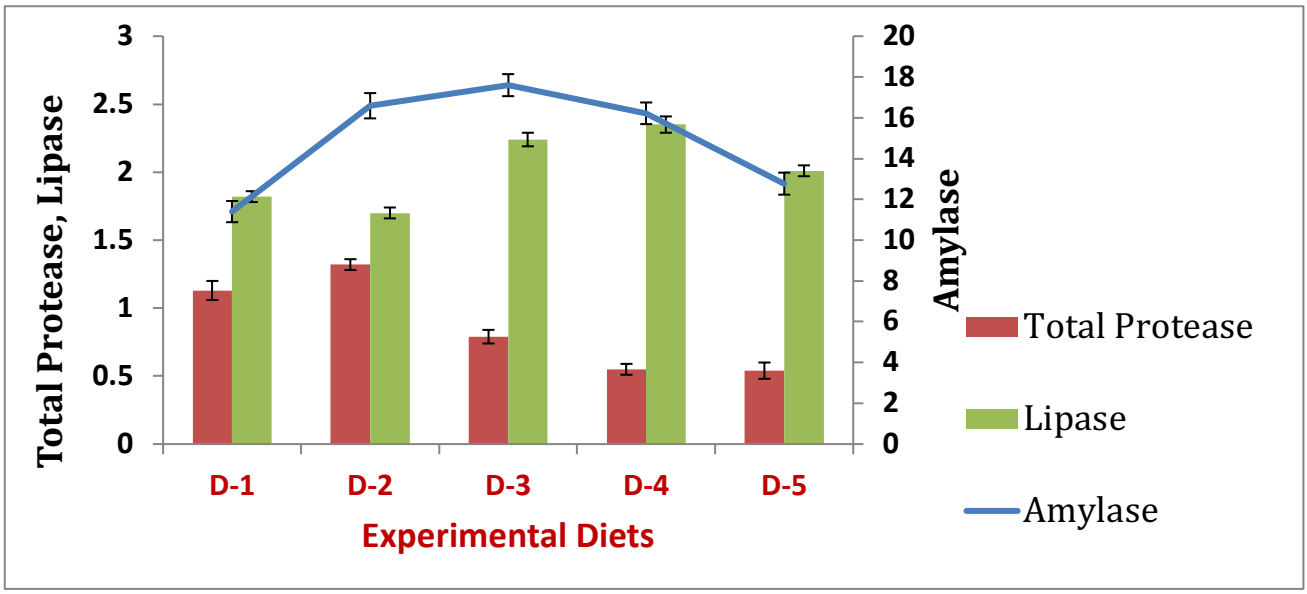
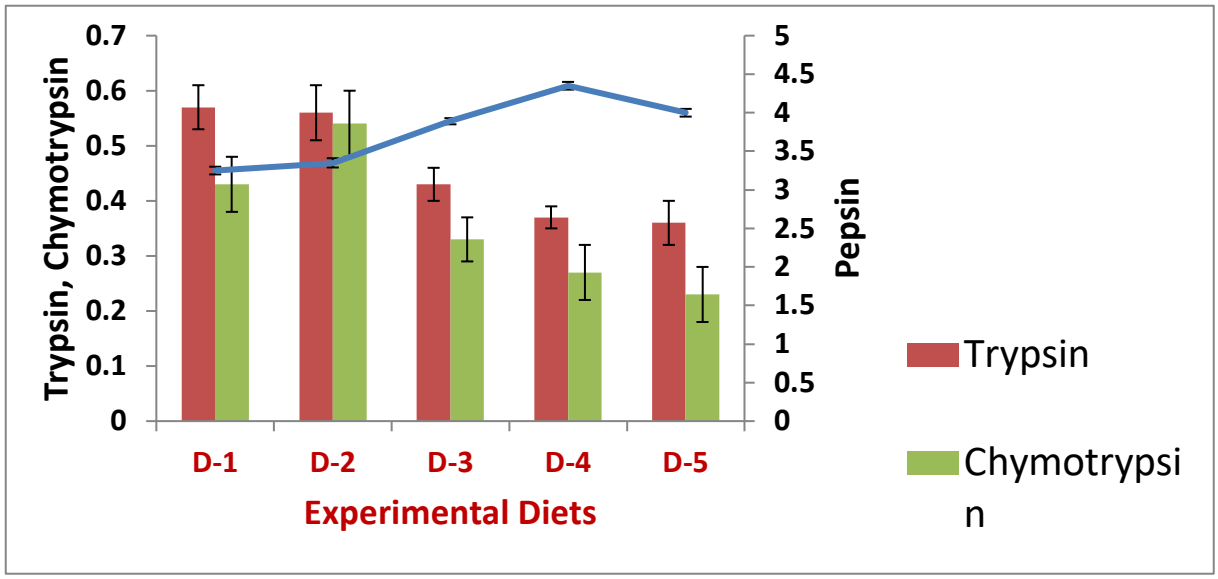


Fig 17. Activity of protein enzymes of *Ompok bimaculatus* fed with different Feed additives



Considering growth performance and survivability of the juveniles, the study suggests that supplementation of egg albumin might be practiced to make the *O. bimaculatus* juveniles adaptive to the formulated diets. The present experiment suggests that a formulated feed having 40% crude protein along with 10% of egg albumin is a better feeding strategy when compared with blood meal, chicken viscera and mussel meat. The study suggests that supplementation of egg albumin might be effective to make the *O. bimaculatus* larvae and juveniles adaptive to the formulated diets for its domestication in freshwater ecosystem..

11. Financial Implications (in Lakhs)

11.1 Expenditure on

(a) Manpower: 33.57 lakhs

(b) Research/Recurring Contingencies: 8.50 lakhs

(c) Non-Recurring Cost (Including cost of equipment): Nil

(d) Any Other Expenditure Incurred: Nil

11.2 Total Expenditure:

Item	Year (1)	Year (2)	Year (3)	Total (Lakhs)
Grand Total	14.19	14.19	13.69	42.07

12. Cumulative Output

a. Special attainments/innovations

1. The proximate and fatty acid composition of *Ompok bimaculatus* (pabda) brood fish, egg and larvae was assayed to have an insight of the nutrient metamorphosis during early life of the species and vis-a-vis parental nutrient composition. The studies regarding nutritional characterization of brood fish, eggs and larvae of *Ompok bimaculatus* revealed that crude protein content was higher in egg whereas crude fat and ash content was higher in pabda brood fish. However, PUFA, EPA and DHA content was higher in larvae.

2. Ontogeny Study of *O. bimaculatus* larvae suggests that the digestive tract of larvae comprised a straight tube dorsally attached to the yolk sac. At 2 dph, mouth opened, teeth were visible and exogenous feeding started. The yolk sac disappeared within 3 dph. Coiling of intestine and presence of stomach were noticed by 8 dph, which was marked with the appearance of pepsin. Activities of the enzymes indicated ontogenetic shift during the period of 8 to 20 dph, which could be associated with changes in food, gut morphology and shift in the activity pattern of digestive and metabolic enzymes. Overall, enzyme activities remained more or less stable beyond 20 dph.

3. A experiment was conducted to study the protein requirement of *O. bimaculatus* larvae 22 for days . The experimental data revealed that 40% crude protein was sufficient for the optimum growth and survival of *O. bimaculatus* larvae and it also recorded that the amylase, lipase and pepsin enzymes was significantly ($P<0.05$) higher in Feed 2 having 40% protein.

4. An experiment was conducted for 42 days to study the lipid requirement of *O. bimaculatus* larvae and it was observed that 8% lipid is sufficient for the optimum growth and survival of *O. bimaculatus* larvae. The activities of digestive enzymes in *O. bimaculatus* were assayed by feeding different levels of lipids. The activities of digestive enzymes in pabda larvae revealed that lipase activity was significantly ($P<0.05$) higher in Feed 2 having 8% lipid.

5. The experiment evaluation of supplementation of different protein sources (w/w basis) suggests that a feed having 40% crude protein along with 10% of egg albumin is a better feeding strategy when compared with blood meal, chicken viscera and mussel meat. The study suggests that supplementation of egg albumin might be effective to make the *O. bimaculatus* larvae and juveniles adaptive to the formulated diets.

b. List of Publications (one copy each to be submitted if not already submitted)

i. Research papers ; 2 nos

Paul, B .N., A. Das, A., R.N. Mandal,R.N., Singh,P., Adhikari, S., Ghosh, K.,Chowdhury,D., Chakrabarti,P.P. and Giri,S.S. (2020) Protein requirement of *Ompok bimaculatus* (Bloch, 1794) larvae. Animal Nutrition and Feed technology (**Communicated)**.

Paul, B .N., A. Das, A., R.N. Mandal,R.N., Singh,P., Adhikari, S., Chakrabarti,P.P. and Giri,S.S. (2020) Proximate and Fatty acid composition of brood fish, egg and larvae of *O.bimaculatus*. Indian Journal of animal Nutrition (**Communicated)**.

i. Reports/Manuals ; 3 training manuals

ii. Working and Concept Papers

iii. Popular articles

iv. Books/Book Chapters: 1

Paul,B.N. and Giri, .S. (2018). Nutrition and Feeding of catfish larvae. S.K.Sahoo, R.Kumar, P.K.Tiwari, B.R.Pillai, S.S.Giri. (Eds.) 2018 Training Manual on Mass Breeding and culture Techniques of Catfishes. SAARC Agriculture Centre, Dakha, Bangladesh pp 73-82.

v. Extension Bulletins

b. Intellectual Property Generation

(Patents - filed/obtained; Copyrights- filed/obtained; Designs- filed/obtained; Registration details of variety/germplasm/accession if any)

c. Presentation in Workshop/Seminars/Symposia/Conferences

(Relevant to the project in which Scientists have participated)

- Paul, B.N**.,** Das, A., Bhowmick, S., Mandal, R.N., S., Singh, Adhikari, S., Ghosh, K., Chowdhury, D. and Chakrabarti, P.P. (2019). Protein Requirement of *Ompok bimaculatus* Larvae. Golden Jubilee International Conference on "Trends in Zoology". January, 03-04, 2019 held at The University of Burdwan, Golapbag, Burdwan, India. Pp 88.

- Chowdhury, D., Paul, B.N. and Ghosh, K. (2019). Profiles of Digestive and Metabolic Enzymes in Butter Catfish, *Ompok bimaculatus* (Bloch, 1794) during early development. Golden Jubilee International Conference on "Trends in Zoology". January, 03-04, 2019 held at The University of Burdwan, Golapbag, Burdwan, India. Pp 90.

- Paul, B.N., Das, A., Mandal, R.N., Singh, P. And Adhikari, S. 2019. Proximate and Fatty acid composition of brood fish, egg and larvae of *Ompok bimaculatus*. Fourth PAF Congress on "Increasing Aquaculture Production in India through Synergetic Approach between Multinational Industries, Domestic Entrepreneurs and Aquaculturists" held at ICAR-CIFA, Bhubaneswar during 15-17 November, 2019. Pp. 27.

- Chowdhury, D., Paul, B.N. and Ghosh, K. 2019. Evaluation of Formulated Diets for Survival, Growth, Digestive Enzymes and Metabolic Functions in Butter Catfish*,* *Ompok bimaculatus* (Bloch) juveniles. International Conference on Animal Nutrition on "Nutritional Strategies for Improving Farm Profitability and Clean Animal Production" held at Biswa Bangla Convention Centre, Kolkata, 17-19 December, 2019.pp 392.

d. Details of technology developed

(Crop-based; Animal-based, including vaccines; Biological – biofertilizer, biopesticide, etc; IT based – database, software; Any other – please specify)

e. Trainings/demonstrations organized

- Organised a Training programme on Applications and Practices of fish feed was organised at RRC, CIFA, Rahara during 23-28 Oct.,2017
- Organised a Training programme on Application of Farm Made Feed for Sustainable Aquaculture Production was organised at RRC, CIFA, Rahara during 03-07 Dec.,2018
- Organised a Training programme on Fish Nutrition and Feeding of Fish at RRC, CIFA, Rahara during 17-21 June ,2019
- Organised a Training programme on 'Captive breeding and seed production of indigenous catfishes with emphasis on *Ompok* and *Mystus* Species' at Kalyani Field Station, RRC Rahara during 30.07.2019 to 03.08.2019.

f. Training received

➢ Mr. Arabinda Das, Scientist received a training on 'Experimental design and statistical data analysis' during January 3-16, 2019 at ICAR-IASRI, New Delhi.

g. Any other relevant information

13. (a) Extent of achievement of objectives and outputs earmarked as per RPP-I

Objective wise	Activity	Envisaged output of monitorable target(s)	Output achieved	Extent of Achievement (%)
1. To study nutrient composition of egg and different stages of larvae	1. Brood stock development of *O.bimaculatus* 2. Production of egg and larvae of *O.bimaculatus* 3. Nutrient composition of egg and larva	Production of Larvae through good brood stock development and production of larvae for experimental work. To know the nutrient profile of brood pabda, egg and larvae.	Sufficient larvae were produced for the experimental work. The nutrient composition data revealed that the crude protein content of brood fish, egg and larvae were 14.4±0.07, 25.90±0.20, 12.23±0.88 (%) respectively and crude protein content was significantly higher in pabda egg.	100%

	4. Collection of Literature, Planning and design of experiment		The fatty acid composition revealed that the saturated fatty acid (SFA) content (% of total fatty acid) of brood pabda, eggs and larvae were 88.48%; 76.04% and 48.60%. The poly unsaturated fatty acid (PUFA) content of brood pabda, eggs and larvae were 4.5%; 10.05% and 33.35% respectively. The EPA and DHA contents were 2.71% and 6.87% in larvae; which was significantly higher in brood fish and egg. It may be summarized crude protein content was higher in egg whereas crude fat and ash content was higher in brood fish. However, PUFA, EPA and DHA content was higher in larvae.	
	Ontogeny and enzyme study	Ontogeny of *O. bimaculatus* was worked out.he yolk sac disappears within 3 days.the overall enzyme activities remained stable after 20days of post hatching.	Ontogeny Study of *O. bimaculatus* larvae: The digestive tract of larvae comprised a straight tube dorsally attached to the yolk sac. At 2 dph, mouth opened, teeth were visible and exogenous feeding started. The yolk sac disappeared within 3 dph. Coiling of intestine and presence of stomach were noticed by 8 dph, which was marked with	100%

			the appearance of pepsin. Activities of the enzymes indicated ontogenetic shift during the period of 8 to 20 dph, which could be associated with changes in food, gut morphology and shift in the activity pattern of digestive and metabolic enzymes. Overall, enzyme activities remained more or less stable beyond 20 dph.	
3.To formulate larval feed and evaluation	1. Feed formulation and Feeding of larvae of *O. bimaculatus* with live and exogenous feed 2. Production of Live food organisms. 3. Monitoring of Water Quality parameters 4. Data analysis and preparation of final report	Development of larval feed for *O.bimaculatus* larvae. A larval feed was developed with 40% protein and 8% lipid. Supplementation of 10% egg albumen (w/w) with the feed should be the feeding protocol for the species to domesticate them in the Aquaculture production system.	(i) **Protein requirement of larvae** An experiment was conducted to study the protein requirement of *O. bimaculatus* larvae for 22 days. During the experiment the water quality parameters were monitored from time to time. The experimental data revealed that 40% crude protein was sufficient for the optimum growth and survival of *O. bimaculatus* larvae and it also recorded that the amylase, lipase and pepsin enzymes was significantly ($P<0.05$) higher in Feed 2 having 40% protein. (ii) **Lipid requirement study of *O. bimaculatus* larvae.** An experiment was conducted 42 days to	100%

			study the lipid requirement of *O. bimaculatus* larvae and it was observed that 8% lipid is sufficient for the optimum growth and survival of pabda larvae. The activities of digestive enzymes in the species were assayed by feeding different levels of lipids. The activities of digestive enzymes in pabda larvae revealed that lipase activity was significantly ($P<0.05$) higher in Feed 3 having 8% lipid. **(iii)Supplementation of live protein supplements** The experimental data suggests that a formulated feed having 40% crude protein along with 10% of egg albumin is a better feeding strategy when compared with blood meal, chicken viscera and mussel meat. Considering growth performance and survivability of the juveniles, the study suggests that supplementation of egg albumin might be practiced to make the *O. bimaculatus* juveniles adaptive to the formulated diets.	

(b) Reasons of shortfall, if any: Nil

14. Efforts made for commercialization/technology transfer

The experimental results obtained are disseminated with the fish famers, fishery extension officers, students, feed manufactures and entrepreneurs in various training programmes organised at RRC, Rahara and its field station at Kalyani, CIFA HQs, farmers field and KVKs. In those training programmes delivered lectures on larval Nutrition, ontogeny of the *O.bimaculatus* larvae and emphasized about the use of precise nutrients(quality fish meal) and fish oil required to prepare the feed for the species.

15. (a) How the output is proposed to be utilized?

The output is proposed to be utilized at farmers field through KVKs, stake holders, feed manufacturers and other fisheries developmental agencies.

(b) How it will help in knowledge creation

The knowledge of ontogeny Study of *O. bimaculatus* larvae was known and it helped to develop the larval feed. The digestive tract of larvae comprised a straight tube dorsally attached to the yolk sac. At 2 dph, mouth opened, teeth were visible and exogenous feeding started. The yolk sac disappeared within 3 dph. Coiling of intestine and presence of stomach were noticed by 8 dph, which was marked with the appearance of pepsin. Activities of the enzymes indicated ontogenetic shift during the period of 8 to 20 dph, which could be associated with changes in food, gut morphology and shift in the activity pattern of digestive and metabolic enzymes. Overall, enzyme activities remained more or less stable beyond 20 dph. The nutrient requirement of larvae was worked out viz., Protein and Lipid required by the species are 40 and 8 (%) respectively. The findings were presented in National and International Seminars and data will also be published in peer reviewed journals.

16. Expected benefits and economic impact(if any)

The feed and feeding protocol of *O. bimaculatus* has been worked out. The larval feed having 40 % protein and 8 % lipid has been developed. As there was no larval feed of *O. bimucalatus*, thus this feed will cater the needs of the sector and help in increasing the butter catfish production in the region.

17. Future line of research work/other identifiable problems

Nutragenomics study on the nutrients of larval feed of butter catfish *O. bimaculatus* would be a line for future research work.

18. Details on the research data (registers and records) generated out of the project deposited with the institute for future use: The register and data generated are maintained and submitted to the Institute in the form of RPF-II and III and Annual Report.

19. Signature of PI, CC-PI(s), all Co-PIs

Project Leader	Co-PI	Co-PI	Co-PI	Co-PI
(Dr. B.N. Paul)	(P.P. Chakrabarti)	(Dr. S. Adhikari)	(Dr. R.N. Mandal)	(Mr. A. Das)

Co-PI

(Dr. K. Ghosh)

20. Signature of Head of Division

21. Observations of PME Cell based on Evaluation of Research Project after Completion

22. Signature (with comments if any along with rating of the project in the scale of 1 to 10 on the overall quality of the work) of JD (R)/ Director

ANNEXURE - VIII

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

(For Guidelines Refer ANNEXURE – XI (H))

PROFORMA FOR RESEARCH PERFORMANCE EVALUATION OF INDIVIDUAL SCIENTIST

23. Institute Project Code * I-95-G

1. Evaluation by PI on the contribution of the team in the project including self

Sl. No.	Name	Status in the project (PI/CC-PI/Co-PI)	*Rating in the scale of 1 to 10
1.	Dr. B.N.Paul	PI	10
2.	Dr.S.Adhikari	Co-PI	10
3.	Dr.P.P.Chakrabarti	Co-PI	10
4.	Dr. R.N Mandal	Co-PI	10
5.	Mr. A. Das	Co-PI	10
6.	Dr. K.Ghosh (Burdwan University)	Co-PI	10

2. Signature of PI

* Individual scientists participating in the project would be assessed for their performance through an appraisal system in a scale of 1 to 10 for each of the following attributes:

No.	Criteria	Marks
1.	Percentage of the assigned activity completed	**40**
2.	Quality of the completed activity	**10**
3.	Authenticity/reliability of the data generated	**10**
4.	Enthusiasm and sincerity to work	**10**
5.	Inferences made	**10**
6.	Collaboration and cooperation demonstrated in performing the task at hand	**10**
7.	Amenability to scientific/academic/laboratory discipline	**10**
	l Score	**100**

ANNEXURE - IX

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

(For Guidelines Refer ANNEXURE – XI (I))

PROFORMA FOR EVALUATION OF A RESEARCH PROJECT AFTER COMPLETION BY PI

10. Institute Project Code

11. Evaluation research project after completion by PI

	Criteria	**Methodology**	**Marks (output)**	**Evaluation by PI**
	Achievements against approved and stipulated outputs under project	**Qualitative and quantitative assessment of objectives and stipulated outputs under the project will be carried out**	**75**	
		a) Activity Input /Projected Output/ Output Achieved	35	**35**
		b) Extent to which standard design methodology, experimental designs, test procedures, analytical methods followed	10	**10**
		c) Does the data justify the conclusions?	05	**05**
		d) Innovativeness and creating of new knowledge	10	**10**
		e) Additional outputs over those stipulated under the project	05	**05**
		f) Creation of linkages for commercialization of technology developed under the project	05	**05**
		g) Is scientific input commensurate to output (manpower, Financial input and time duration)?	05	**05**
	Publication/ awards	A essment will be done in respect of: Research papers; Reports/Manuals; Working and Concept Papers; Books/Book Chapters/Bulletins. Quality of publication (s) and Awards /Scientific recognitions received llowing publications were made during the project work: Research papers-2 (communicated to peer reviewed journals), Book chapter -1, Training Manual-3 nos. and Conference/Seminar presentations 4. (International -3 and National level -1)	**10**	**10**

	Additional facilities created	Facilities created in terms of laboratory. Research set-up, instrumentation, etc. during the project. Fatty acid analysis facility was created in the Laboratory with preparation of Fatty Acid Methyl Ester and analysis in gas Chromatography. institute collaboration was developed with University of Burdwan where Dr. Koushik Ghosh was the collaborator. Ontogeny study protocol of *O.bimaculatus* was developed along with study of enzymes during early stage of larvae in collaboration with University of Burdwan. Feeding protocol *O.bimaculatus* was worked out in the project.	**05**	**05**
	Human Resource Development (Scientific and Technical)	Scientist trained in different areas Arabinda Das, Scientist trained on a Programme 'Experimental design and statistical data analysis' during January 3-16, 2019 at ICAR-IASRI , New Delhi. B.N.Paul, attended two International and one National Conferences, Dr. R.N.Mandal attended one International conference. B.N.Paul and Mr. A.Das attended one National Conference. All the Scientist participated and presented paper in the above seminars during technical discussions.	**05**	**05**
	Revenue generated under the project/ avenues created for revenue generation	Resources and revenues generated different training programmes were conducted 1.Organised a Training programme on Applications and Practices of fish feed was organised at RRC, CIFA, Rahara during 23-28 Oct.,2017 2.Organised a Training programme on Application of Farm Made Feed for Sustainable Aquaculture Production was organised at RRC, CIFA, Rahara during 03-07 Dec.,2018 3.Organised a Training programme on Fish Nutrition and Feeding of Fish at RRC, CIFA, Rahara during 17-21 June ,2019 Revenue generated from the training programme were 2.04 Lakhs	**05**	**05**

	Product/Process/Technology/ IPR / commercial value of the technology developed	Details to be provided on a) Products: The larval feed was developed for *O.bimaculatus* larvae. b) Process: Preparation of larval feed process was developed with different quality fish meal and fish oils alonf with other feed ingredients. c) Technology: Feed and feeding protocol was developed for the *O.bimaculatus* larvae d) IPR e) Registration of the varieties	**10**	**06**
	Quality of available documents of the project duly authenticated	Research Project Files, Data, Reports etc. records are available with the Centre	**05**	**05**
Total Marks			**115**	**111**
	Time lines of execution of the project	Marks will be deducted if extension sought over the approved project duration beyond recorded and officially granted extension with recorded reasons		
		Up to 5%		
		Up to 10%		
		Up to 30 %		
		Beyond 30 %		
Score: Score obtained to be counted out of 100 to compensate for activities not relevant to the project				

However, looking into the requirements of different research institutes and disciplines, IRC may modify the indicators, their weights and total scores. The time gap for assessment of different indicators may also be decided by IRC

12. Signature of PI

भाकृअनुप
ICAR

ICAR-Central Institute of Freshwater Aquaculture
(ISO 9001:2015 Certified Institute)
(Indian Council of Agricultural Research)
RRC,Rahara, 700118,West Bengal

CIFA