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Evaluation of preference of dry feed, bio-encapsulated and non-bio-encapsulated live feed and survival of the walking catfish, *Clarias batrachus* (L.) juveniles

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Abstract
Feed preference of *Clarias batrachus* juveniles (average weight 0.69±0.01 g) was observed with commercial feed (CP 9910-L), artificial feed (laboratory made), and live feed either non-bio-encapsulated (NBE) or bio-encapsulated with a presumptive probiotic bacterium (BEP), *Bacillus aryabhattai* KP784311 isolated from the gut of adult *C. batrachus*. Altogether 360 juveniles (20-day old) were distributed in six dietary treatments: T1 (C.P. 9910-L), T2 (artificial feed), T3 (NBE *Tubifex*), T4 (NBE chironomid larvae), T5 (BEP *Tubifex*) and T6 (BEP chironomid larvae) keeping 20 in each tank in triplicate. The catfish juveniles were fed twice at 5% of body weight day^{-1} for 10 days and quantity of uneaten feed measured after 2 h of serving to determine the feed preference. Results revealed least preference in T2, and maximum in T4 and T6 in comparison to the other groups. Further, the results suggested that *C. batrachus* juveniles preferred chironomid larvae more than *Tubifex* and even bio-encapsulation did not hinder acceptability of the midge larvae. Feeding on chironomid larvae in BEP group (T6) resulted in highest survival (90±1%) in contrast to other groups. The investigation with probiotic-encapsulated chironomid larvae may be considered as a model to evaluate efficacy of the probiotics for catfish juveniles.

Keywords: *Bacillus*, bio-encapsulation, probiotic, *Clarias batrachus*, chironomid larvae, *Tubifex*

1. Introduction
Fish juveniles prefer live feed to formulated artificial feed as they lack well developed digestive system to process formulated diet [1]. Rotifers (*Brachionus plicatilis*), *Artemia*, and micro-algae have been commonly used as live or natural feed for catfish juveniles [2]. However, utilization of chironomid larvae or *Tubifex* as live feed is scarce in the culture of catfish juveniles. Although artificial feed might fulfil nutritional requirements of the juveniles for digestibility, proper growth etc. but attention have not been paid much in this area [3].
The freshwater walking catfish, *Clarias batrachus* is a voracious and opportunistic feeder consuming a wide variety of prey including eggs and larvae of other fishes, small fishes, a number of invertebrates including arthropods like crustaceans and insects and occasionally plant materials [4]. The *C. batrachus* is vastly appreciated for its high market value, recuperative power and delicacy. Fish farmers are taking much attention for its high consumer preference and have been expanding the culture practice with this species since last few decades [5]. Availability of healthy fish juveniles is the basic requirement for successful intensive fish culture. Unfortunately, the population of *C. batrachus* is being deteriorated due to various anthropogenic and natural reasons [6]. Natural resources of *Clarias* seed have sharply declined due to diverse ecological imbalance in their natural breeding ground [7]. Moreover, collection of healthy juveniles from natural sources is getting difficult due to their high mortality caused by lack of optimum level of live feeds and proper nutrition. Thus, non-availability of juveniles during the stocking time seems to be the main hurdle for its commercial culture [5]. However, mortality of catfish juveniles might be reduced by making adequate amount of nutritious feed available to them. Therefore, selection of suitable feed could be viewed as an important prerequisite to ensure survivability and growth of the juveniles for commercial production of the species [8].
This preliminary investigation is aimed at evaluation of feed preference and survival of the juveniles of *C. batrachus*. Appropriate strategy for likely manipulation of feed has been made

through comparison of commercial and formulated dry feeds with probiotic-encapsulated and non-encapsulated live feeds for the juveniles.

2. Materials and Methods

2.1 Collection of juveniles and maintenance in the laboratory

Fifteen day old juveniles of *C. batrachus* were obtained from a reputed hatchery, Dhibar Fish Farm, Ramsagar (23°09′ N, 87°27′E), Bankura, West Bengal, India and were kept in fiber reinforced plastic (FRP) tanks (45L) with supply of non-chlorinated fresh tap water and continuous aeration. They were subjected to acclimatization in the laboratory condition for 5 days with feeding of zooplanktons taken from pond. Water parameters, like temperature, dissolved oxygen and pH were monitored regularly with the use of PCS TEST-35 multi parameter and dissolved oxygen by Fisher Scientific Traceable Portable DO Meter (Model no. 0666266) to make sure that they are within tolerance limits of the experimental species. Fecal matter and debris were siphoned out daily to ensure clean and fresh water inside the tank.

2.2 Experimental trial

After completion of acclimatization, the experimental trial was conducted for 10 days in the laboratory condition. Altogether 360 juveniles were randomly distributed in six dietary treatment groups in three replicates, thus each FRP tank was stocked with twenty fish juveniles of average weight 0.69±0.01g. Among the experimental groups, T1 and T2 were provided with commercial feed (CP 9910-L, crude protein: 30%) and artificial feed (crude protein: 30%) prepared in the laboratory respectively. The groups T3 and T4 were fed with non-bio-encapsulated *Tubifex* and chironomid larvae respectively. Correspondingly, bio-encapsulated *Tubifex* and chironomid larvae were fed to the fish reared as groups T5 and T6. During the experimental period the fish were fed at 5% of body weight day^{-1} in two equal proportions (9.00h and 16.00h) as followed elsewhere for *Clarias* sp.[9, 10]. *Tubifex* and chironomid larvae were procured from the nearby canals and were maintained in the laboratory for its use as food of the growing juveniles. Daily ration for the live feed was adjusted on dry weight basis.

2.3 Preparation of bio-encapsulated feed

The putative probiotic bacterium, *Bacillus aryabhattai* KP784311 was isolated from the gut of adult *C. batrachus*. Isolation and likely beneficial attributes of the bacterium has been stated in the previous study [11]. Bio-encapsulation of live feed organisms was carried out by suspending *Tubifex* and chironomid larvae in bacterial suspension prepared at concentration of 1×10^7 CFU mL^{-1} and incubated at 25 °C for 4 h separately. The bacterial suspension was prepared following Akbar *et al.* [12] with minor modification. Prior to encapsulation, the live organisms were kept for 12 h in sterilized distilled water treated with broad spectrum antibiotics (10µg ml^{-1}, kanamycin and rifampicin) for elimination of bacteria associated with them. Preparation of presumptive probiotic encapsulated live feed was done daily in order to maintain the concentration of the probiotic bacteria in feed. Live feed organisms were incubated in bacterial suspension for 2 h, 4 h, and 6 h at 25 °C in order to observe successful encapsulation. Suspended and incubated organisms of respective time durations were processed after Basu *et al.* [13], aseptically on sterilized tryptone soya agar (TSA) (Himedia, Mumbai, India) plates within laminar airflow and kept in BOD incubator for overnight at 30 °C. Colony forming units (CFU)/mL were calculated for each time duration.

2.4 Statistical analysis

$$\text{Specific Growth Rate (SGR)} = 100\left[\frac{\ln W_f - \ln W_i}{T}\right]$$

Where W_f and W_i are the final and initial wet weights of fish respectively;

T is the trial period in days.

Survival Rate = (Final number of juveniles/ Initial number of juveniles) × 100

Growth data (Length, weight and SGR) was analyzed using one-way analysis of variance (ANOVA) and a post hoc analysis (Tukey HSD) followed by Zar [14] using Excel 2013. The data of survival and specific growth rate are presented as mean ± SD (standard deviation) of three replicate groups per treatment (n = 3).

3. Results

Following incubation with the presumptive probiotic bacterium (*Bacillus aryabhattai* KP784311), CFU count of the live feed associated bacteria varied between 2 and 4 hours of incubation while it was almost equal at 4 and 6 hours of the same. Therefore, incubation for bio-encapsulation of the live feed was done at 25 °C for 4 h (Table 1). Amongst the different types of tested feed, preference of the feed item as evidenced through the amount of unconsumed feed was in the sequence of: T6>T4>T5>T3>T1>T2 (Figure 1). Maximum growth and survivability were associated with maximum intake of feed. In the T6 group final length, final weight, survival rate and specific growth rate were found to be higher than that of other groups. Data on growth performance and survivability are presented in table 2.

Table 1: Optimization of bio-encapsulation of live feed organisms with the presumptive probiotic bacterium, *Bacillus aryabhattai* KP784311

Type of live feed organisms	Duration of incubation (h)	Temperature set (°C)	Colony forming unit (CFU)/ml
Chironomid midge larvae	2	25	0.3×10^8
	4	25	1×10^8
	6	25	1.1×10^8
Tubifex	2	25	0.2×10^8
	4	25	1×10^8
	6	25	1×10^8

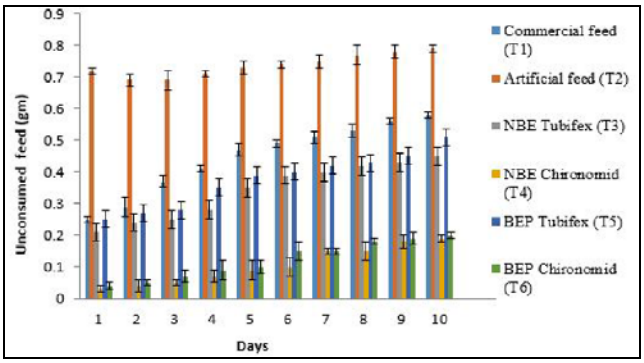

Fig 1: Unconsumed feed in six different experimental groups of *C. batrachus* juveniles.

Table 2: Growth performance and survivability of *C. batrachus* juveniles at different feeding regimes.

Treatments	Parameters					
	Initial length (cm)	Final length (cm)	Initial weight (gm)	Final weight (gm)	Survival rate (%)	Specific Growth Rate (% day^{-1}/ fish)
T1	2.55±0.02^{b}	3.06±0.02^{d}	0.69±0.01^{b}	1.65±0.03^{d}	74±1^{d}	3.5±0.15^{d}
T2	2.52±0.01^{d}	3.01±0.01^{e}	0.66±0.01^{c}	1.62±0.04^{e}	70±1^{e}	1.8±0.1^{e}
T3(NBE)	2.54±0.01^{c}	3.09±0.01^{d}	0.69±0.09^{b}	1.68±0.01^{c}	81±1^{c}	5.3±0.1^{c}
T4(NBE)	2.55±0.01^{b}	3.12±0.02^{c}	0.69±0.06^{b}	1.72±0.03^{a}	84±1.52^{b}	6.5±0.15^{b}
T5(BEP)	2.55±0.01^{b}	3.19±0.01^{b}	0.7±0.1^{a}	1.70±0.01^{b}	80±1^{c}	5.6±0.15^{c}
T6(BEP)	2.56±0.02^{a}	3.24±0.03^{a}	0.71±0.01^{a}	1.72±0.01^{a}	90±1^{a}	7.7±0.1^{a}

Results are mean ± standard deviation of 3 determinations
Values with the same superscripts in the same vertical column are not significantly different (P<0.05)

4. Discussion

Dependence on live feed at the early stages of development has been established in diverse species of carps [15, 16] and catfishes [2, 17, 18]. Along with the various zooplanktons, aquatic culturable species have been tested with *Artemia* naupli in captive rearing of juvenile stages [19, 20]. In contrast, use of *Tubifex* and midge larvae for captive rearing of early stages of catfish has been either rarely or not even addressed. Although midge larvae have been documented as an ideal food source for the fish juveniles due to presence of most of the major nutrients in adequate amount [21], their utilization is restricted due to lack of established culture conditions [22]. However, limited success in this regard has shown promising in utilization as live feed for captive rearing of fish [23]. Therefore, the present study with midge larvae and *Tubifex* as live feed organisms is to assess acceptability of the catfish juveniles. Apart from supply of nutrients in adequate amount, supplementation of the probiotics has come out to improve nutrient utilization and disease resistance and also as an effective strategy to ensure growth and survivability in juvenile stages. However, suitable delivery method is to be ensured for supply of probiotics to the target fish. The autochthonous putative probiotic strain, *Bacillus aryabhattai* KP784311 isolated from the foregut of adult *C. batrachus* had been illustrated as a potent extracellular enzymes *viz*. protease, amylase and lipase producing strain in the previous study [11]. In the present study catfish juveniles have been found to prefer chironomid midge larvae than to *Tubifex*. It is evident from this study that bio-encapsulation did not hamper their acceptability of feed rather resulted in better survival. In recent years, much importance has been given to improve the nutritional status of live feed with various techniques of bio-encapsulation [24]. Proficient growth and production of fish depend on feeding of the best possible diets at levels not beyond the dietary needs [25]. Higher mortality of catfish juveniles due to use of commercial and artificial feed has established that prepared feed does not suit to them. Among the fish species, *C. batrachus* requires live feed during their juvenile stage. Many factors are accountable influencing prey selection by juveniles and these include prey size, density, and motion [26-28]. Fish juveniles are engrossed to living prey by their movement [29]. Not only the characteristics of the prey, selection of feed also depends on the juvenile characters such as, sensory capabilities, previous experience, motor ability, mouth gape and body size [30, 31]. Many investigations regarding live feed selection concentrate on the relationship between prey size and mouth size as the primary determinant of feed selection [32, 33]. Live feeds contain protein rich nutrition that leads to successful intensive catfish culture. Suitability of live feed for catfish juveniles could also be because of their high digestibility. The wriggling movement of solitary chironomid larvae might seem to be attractive to the juveniles. So the catfish juveniles easily prey upon the chironomid larvae which represent a major group of benthos insects in freshwater ecosystem [34]. According to De La Noue [35] chironomid larvae are with relatively high protein content (56%) and their digestibility is also relatively high (73.6%). Moreover utilization of midge larvae in commercial aquaculture leads to promote growth in young carnivorous fish [36]. On the other hand, *Tubifex* are long and usually form clump by aggregation. On account of clustering, it appears large sized object that is more likely to ignore for its difficulty to engulf. However, in many studies *Tubifex* was used in juvenile rearing of European catfish *Silurus glanis* [37] and Asian catfish *Pangasius bocourti* [38]. Though the main purpose of this experiment was to observe the feed preference among the catfish juveniles, it has also been noticed that administration of bio-encapsulated live feed in the catfish juvenile rearing has increased both growth and survival leaving water physico-chemical parameters unaffected as indicated elsewhere [39]. However, it is worthy to mention that an observation based on duration of only 10 days may not be adequate to comment on the growth response of the catfish juveniles. Therefore, forthcoming studies should be conducted with longer duration and in field condition to conclude the effects of bio-encapsulated live feed on growth performance of the catfish juveniles.

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6. Conflicts of Interest

We, authors of this article, declare no conflict of interest.

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