International Journal of Fisheries and Aquatic Studies 2014; 2(2): 142-144

ISSN: 2347-5129
IJFAS 2014; 2(2): 142-144

www.fisheriesjournal.com
Received: 20-09-2014
Accepted: 06-10-2014

Manoranjan Paramanik
Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751 002, Odisha, India.

Shahajahan Ferosekhan
Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751 002, Odisha, India.

Sangram K. Sahoo
Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751 002, Odisha, India.

Does the dark condition enhance growth and survival of *Clarias batrachus* larvae at higher stocking density?

Manoranjan Paramanik, Shahajahan Ferosekhan and Sangram K. Sahoo

Abstract
An experiment was conducted to rear the larvae in total dark environment with a probability of enhanced growth and survival, as the *Clarias batrachus* larvae is photonegative in nature. Hence the larvae were reared at four density treatments (D_{1000}, D_{2000}, D_{3000} and D_{4000}) with three replications of each treatment for 14 days under dark condition to find out the optimum density level during its rearing. Growth parameters such as final total length and wet weight of the *C. batrachus* larvae were recorded significantly ($P < 0.05$) higher in D_{1000} followed by D_{2000} treatment group and it was observed that the growth rate decreased with the increasing level of stocking density. The daily weigh gain, weight gain, percent weight gain and specific growth rate were found significantly ($P < 0.05$) higher in D_{1000} treatment group. The survival rate was recorded more than 90% in D_{1000} and D_{2000} treatment groups at the end of experiment and among all the treatment groups D_{1000} showed significantly ($P < 0.05$) higher survival rate. The lowest survival rate was observed in higher stocking density treatment group (D_{4000}). If growth and survival of *Clarias batrachus* larvae is considered, up to 2000 larvae m^{-2} density is acceptable while rearing in dark condition.

Keywords: *Clarias batrachus*, dark environment, growth, survival

1. Introduction
The declining production of fish from natural water bodies becomes a real challenge to aquaculturists to provide fish protein to the increased human population. Hence, utilisation of water bodies with species diversification along with simple and low-cost techniques may be the fore-runner activities for the enhanced fish production in future times. *Clarias batrachus* as a potential catfish is commonly cultured in India and South-East Asian countries [2, 16]. This catfish is one of the acceptable species among all other catfishes due to high consumer preference because of its delicacy and medicinal values [7]. Supply of adequate quantity and desired size of seed is still a constraint for *C. batrachus* aquaculture in Indian sub-continent. Even though the seed rearing technology is standardised to many extents but the growth rate of larvae is slow in culture condition. But many abiotic and biotic factors like light regime, shelter, density, environment and their interactions have a scope for growth parameters [5]. Growth and survival of *C. batrachus* and *C. gariepinus* have strongly influenced by stocking density [8, 12]. Photoperiod is one of the crucial physical factors, which enhances growth and survival of fish larvae [3]. Extended photoperiod enhances growth in *Psetta maeotica* [15], whereas low intensity of light enhances growth in *C. gariepinus* fingerlings [8]. The larvae of *C. gariepinus* also perform well at low density in combination with low intensity of light [9]. The larvae of *C. batrachus* remain in clusters in the rearing tanks during day time, indicating its photo negative behaviour. Hence, the possibility of rearing the larvae in dark condition may improve the performance, profitability of a hatchery and sustain the *C. batrachus* seed production. So, the present study was conducted to evaluate the possibility of enhanced growth and survival of *C. batrachus* under the dark rearing condition in combination with different stocking density.

2. Materials and Methods
The larvae were obtained by induced breeding of *C. batrachus* following a standard method [13]. The newly hatched larvae were kept in rearing tanks for three days till the yolk sac was completely absorbed. The four days old larvae were used for the experimental purpose.

Correspondence:
Sangram K. Sahoo
Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751 002, Odisha, India.

The initial wet weight and total length of twenty larvae were recorded through electronic weighing balance with 0.0001 mg precision and standard measuring board, respectively. Four different stocking density levels (1000, 2000, 3000 and 4000 nos. m^{-2}) were maintained and each stocking density level represents four treatment groups (D_{1000}, D_{2000}, D_{3000} and D_{4000}). Triplicate fibre glass tanks (1 m x 0.5 m) were used for each treatment group and desired quantity of larvae was stocked as per the density level in each tank. Six inch water depth was maintained uniformly in each treatment tank. All the tanks were kept under dark environment by putting a black polythene sheet over the tanks with the bamboo pole support, ensuring air passage and complete dark environment. The tanks were also provided with aeration round the clock. All the treatment tanks were cleaned twice daily replacing two third of water volume within 30-40 minutes. The larvae were fed live mixed plankton just after the refilling of water. The water quality parameters were analysed 4-5 days interval following the standard procedure [4]. Twenty five larvae were taken from each treatment tank for sampling at 7th and 14th days to record the total length and weight. The sampled larvae were brought back and released in their respective tanks. At the end of the experiment (day 14), all the survived larvae were counted and recorded.

One way Analysis of variance (ANOVA) was performed using the IBM SPSS (Statistical software, Version 20.0). Duncan's multiple comparison tests was used to find differences between treatment means [14]. All the results were expressed as means ± standard deviation. For all treatment comparisons, $P < 0.05$ was accepted as the significant level.

3. Results and discussion

The water quality parameters were depicted in Table 1. The water quality of different treatments was within the normal range for rearing of fish species [4]. These were also within the permissible range as observed while rearing other catfishes in the hatchery condition [10, 11]. This could be due to regular water exchange and provision of continuous aeration. The rearing performance of *C. batrachus* larvae in all treatment groups (D_{1000}, D_{2000}, D_{3000} and D_{4000}) is reflected in Table 2.

Table 1: Variations of water quality parameters (Mean ± S.D) in the tanks used for rearing of *C. batrachus* larvae at different treatments under dark condition during two weeks period

Parameters	D_{1000}	D_{2000}	D_{3000}	D_{4000}
Temperature (ºC)	28.33±0.304	28.46±0.305	28.66±0.305	28.53±0.577
pH	7.53±0.305	7.54±0.115	7.60±0.200	7.26±0.115
Dissolved oxygen (mg L^{-1})	5.86±0.306	5.66±0.230	5.33±0.115	5.20±0.200
Alkalinity (mg L^{-1})	126±5.291	128±2.000	120±5.291	122±4.000
Ammonia (mg L^{-1})	0.00267±0.0005	0.00261±0.0004	0.00274±0.0005	0.00281±0.0005

Table 2: Effect of stocking density on *C. batrachus* larval growth and survival under dark rearing condition over two weeks rearing period. Data are based on mean ± SD of triplicate tanks for each treatment level.

Parameters	D_{1000}	D_{2000}	D_{3000}	D_{4000}
Initial Length (mm)	7.80±0.015	7.80±0.015	7.80±0.015	7.80±0.015
7th day length (mm)	14.76±0.550 ^{c}	13.13±0.550 ^{b}	13.00±0.100 ^{b}	12.10±0.173 ^{a}
Final Length (mm)	17.83±0.153 ^{c}	14.67±0.666 ^{b}	13±0.656 ^{a}	12.87±0.351 ^{a}
Initial Weight (mg)	4.41±0.025	4.41±0.025	4.41±0.025	4.41±0.025
7th day weight (mg)	19.23±0.481^{c}	11.87±0.192 ^{b}	10.64±0.791 ^{a}	10.02±0.298 ^{a}
Final Weight (mg)	39.47±0.829 ^{c}	22.41±2.565 ^{b}	13.59±1.3 ^{a}	12.01±0.501 ^{a}
Weight gain (mg)	35.06±0.813 ^{c}	18.00±2.564 ^{b}	9.18±1.30 ^{a}	7.60±0.510 ^{a}
Daily weight gain (mg)	2.5±0.057 ^{c}	1.29±0.180 ^{b}	0.65±0.095 ^{a}	0.55±0.038 ^{a}
Weight gain (%)	795.01±18.789 ^{c}	408.24±58.158 ^{b}	208.09±29.476 ^{a}	172.41±11.554 ^{a}
Specific growth rate (%)	16.87±0.161 ^{c}	12.47±0.914 ^{b}	8.63±0.735 ^{a}	7.70±0.327 ^{a}
Survival rate (%)	91.67±2.369 ^{bc}	93.87±3.073 ^{c}	84.67±6.093 ^{b}	71.52±2.965 ^{a}

$^{a-c}$ Mean values in a row with different superscripts are significantly different (*P<0.05)*
Weight gain= (Final weight-initial weight)/days of experiment; Percent weight gain= (Final weight-initial weight)/ Final weight x 100;
Specific growth rate= (ln Final weight-ln Initial weight)/Initial weight x100

The growth parameters (length and weight) of D_{1000}, D_{2000}, D_{3000} and D_{4000} treatment groups indicated a declining trend as the density increased. The significantly ($P < 0.05$) higher total length and wet weight was recorded in D_{1000} treatment group compared to all other treatment groups. This was due to better feed utilisation and lower level of competition among the larvae for food in the D_{1000}. Similar results were also reported for *C. batrachus* and *C. gariepinus* rearing in hatchery condition [12, 9]. If growth is considered, then the density of this species can be enhanced maximum to 2000 m^{-2}, after which the growth trend reduced consistently in the dark rearing condition. The larvae of *C. batrachus* usually show resting behaviour or remain in cluster most of the time during their rearing in normal photoperiod, indicating its photonegative nature. But it was observed that the larvae remain spreaded and active throughout while rearing in dark environment, satisfying its behaviour. Hence, maximum energy might have expended on the metabolic activity rather than conservation, resulting lower growth. Therefore, the chance of getting good growth in complete darkness is meagre. In contrast, the positive trend of growth in *C. gariepinus* early stage and juveniles was reported, indicating beneficial effect of rearing in dark condition [1, 6]. The reason for non-response for better growth of *C. batrachus* larvae in total dark rearing condition is unanswered at this time. Is it due to larval stage of *C. batrachus* or complete dark rearing environment? Will it be effective in dim light as reported in *C. gariepinus* [9]? Hence, further study on rearing of different life stages in combination with intensity of darkness may indicate the possibility. Further the colouration of *C. gariepinus* becomes darker in dark environment with better growth compared to the lighter colour in normal photoperiod [10], indicating its photophobic nature. But we could not see any changes in body colouration of larvae during their rearing in the darkness. The brownish colour of larvae in darkness was also similar as usually seen in the larvae reared in normal photoperiod. Hence, the unchanged colour of *C. batrachus* larvae was probably due to non-response to complete dark environment. The daily weight gain, weight gain and specific growth rate considered as growth indicators were observed significantly lower in D_{3000} and D_{4000} treatment group compared to other two density levels. This was due to poor growth performance of larvae during their rearing at higher density. The survival rate of D_{1000} and D_{2000} treatment groups were recorded more than 90% and it decreased significantly ($P < 0.05$) in D_{3000} and D_{4000} treatment group. This higher mortality at D_{3000} and D_{4000} treatment groups might be due to crowding effect and feed disparity among the larvae during rearing in dark environment. This observation of lower survival was reported on *C. batrachus* larval rearing in hatchery condition at higher density [12]. The rearing in dark environment did not help much to get higher survival in the present study compared to *C. gariepinus* rearing in dark [9]. This higher survival of *C. gariepinus* could be due to behavioural difference with *C. batrachus*, which may be considered as species specific. They show active movement in dark compared to resting condition in light environment. Hence, the cannibals get better opportunity to prey on the resting fish rather than active fish, resulting in higher survival during dark rearing of *C. gariepinus*. So providing dark environment is considered as one of the management strategy to enhance its survival during captive rearing. But providing complete dark environment is not beneficial during rearing of *C. batrachus* larvae. Hence, providing shelter as well as quantification of shelter in the rearing tanks must be evaluated to explore the growth parameters in normal photoperiod. Further evaluation of minimal intensity of light thresh-hold for the *C. batrachus* larvae may answer some unanswered questions in this article.

4. Acknowledgement
The first author expresses his sincere thanks to Dr B.K. Das and Director, CIFA for their support and encouragement during conducting this experiment under pre-Ph.D work.

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