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Growth Performance of *Hypophthalmichthys molitrix* and *Barbodes gonionotus* Fingerlings by Feeding Microalgae Cultured on Fertilizer Factory Effluent

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Abstract

Growth performance of *Hypophthalmichthys molitrix* (Silver carp) and *Barbodes gonionotus* (Sar puti) fingerlings were studied separately in aquaria by feeding cultured microalgae (*Chlorella ellipsoidea, Scenedesmus obliquus* and *Spirulina platensis*) in different combinations for a period of 21 days. Fertilizer factory effluent was used to grow these microalgae. Significantly ($p<0.05$) higher weight gain of *H. molitrix* (81.16%), was in the treatment T_2 (Rearing by feeding 100% *S. platensis*) and the minimum percentage (- 4.29%) was obtained in the control (fed on fishmeal and mustered oil cake). Specific growth rate (SGR, %/day) of *H. molitrix* were determined - 0.21 to 2.84 for all the treatments which was significantly ($p<0.05$) higher in T_2. Survival rate of *H. molitrix* was 25.90 to 77.80%. In the case of *B. gonionotus*, significantly ($p<0.05$) higher weight gain (149.10%) was observed in t_2 (Rearing by feeding 100% *S. platensis*). The SGR (%/day) of *B. gonionotus* were ranged from 2.25 to 4.33 which was significantly ($p<0.05$) higher in t_2 than all other treatment except control. Survival rate of *B. gonionotus* was ranged from 97.78% to 100%. The reared fingerlings were found nutritionally rich.

Key words: Fingerlings, Microalgae, Growth performance, Specific growth rate.

Introduction

Hypophthalmichthys molitrix and *Barbodes gonionotus* are exotic fish, which are locally known as silver carp and Thai sharpunti, respectively. They are very popular as table fish in Bangladesh for their palatability and good taste, and their fast growth and high yield. As they become marketable size within short time, fish farmers prefer to these species. So, the demand of fry and fingerlings of these species are increasing day by day. Though, the seed production increasing now a days but the supply of fingerlings are not sufficient, as the rearing technique of this stage is not improved due to appropriate feed resulting high mortality. To make fish fingerlings readily available to the fish farmers, we need to develop an appropriate technology for large-scale production of fish fingerlings. Feed cost is one of the most important largest operational costs in aquaculture (De Silva and Davy, 1992). It is very difficult to supply of natural live food when large quantities of fish fry are reared. It is interesting to note that fish are fed higher precentage of protein in their diet than land animals (Lovell, 1991). The alga, *Chlorella* contains long chain polyunsaturated fatty acids (PUFA), which make it a valuable food for marine invertebrates and fish (Watanabe *et al.*, 1983). It is recorded that the presence of *Chlorella* improved the growth and survival of 40 species of fishes studied (Jhons, 1970). The protein content of *Chlorella* is appreciable higher (50% of the dry weight) than that of the best vegetable sources of protein used in animal feed (Bhanou and Vass 1973). According to Yap *et al.* (1982) still the highest proportion of the protein (33%) can be replaced by *Spirulina* or *Chlorella* without negative symptoms. Microalgae have been used as live food in intensive hatchery systems (Walne, 1981). Microalgae not only play an important role in aquaculture as a food source but together with bacteria they also have an important role in dissolved oxygen and carbon dioxide balance in the water (Pruder, 1983). Successful laboratory rearing will help to develop culture techniques of the fish fingerlings. Therefore, considering all these in mind,, the present study was undertaken to rear *H. molitrix* and *B. gonionotus* fingerlings in laboratory condition feeding cultured microalgae and to analyze the nutritional values of the fishes.

Materials and Methods

Preparation of Media and Culture of Microalgae

Fertilizer factory effluent (FFE) was used to grow the *Chlorella ellipsoidea, Scenedesmus obliquus* and *Spirulina*

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platensis. Among the three microalgae *Chlorella ellipsoidea* and *Scenedesmus obliquus* that was used for mass culture was isolated earler and pure culture was mainained in the laboratory (Live Food Culture Laboratory, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh) by authors. On the other hand *Spirulina platensis* was initially collected from the University of Putra, Malaysia and pure culture was maintained by the authors in the same laboratory. The FFE was collected from Jamuna Fertilizer Factory (urea), Jamalpur. The collceted FFE was diluted at 50% level by tap water and decomposed for a period of 15 days by aeration. Hundred-liter capacity aquarium was used for the continuous culture of *C. ellipsoidea, S. obliquus* and *S. platensis* in prepared FFE medium. The aquaria were set in the balcony of Live food Culture Laboratory under natural light and temperature. Six aquaria were set for each species of microalgae. After successful growth of microalgae rearing of fingerlings were started. The same amount of FFE medium was added regularly in the aquaria which were removed at the time of collection of microalgae for fingerlings. Prior to rearing of fingerlings the proximate composition of cultured algae were analyzed (Horwitx, 1984).

Culture of Fish Fingerlings

Culture of Fish Fingerlings was performed in two steps for a period of three weeks of each species in the Live Food Culture Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University Mymensingh. In the first step, attempt was taken to rear *H. molitrix* (where the treatments are mentioned as T_1 - T_8) and in the second step, attempt was made to rear *B. gonionotus* (where the treatments are mentioned as T_1 -T_8) in aquaria. In both steps of experiment, the fingerlings were fed with cultured microalgae (*C. ellipsoidea, S. obliquus* and *S. platensis*) of seven different combinations (Table I) having control (feeding fishmeal and mustered oil cake). The feeds were supplied twice daily at the rate of 5% of body weight of the fingerlings in both steps of experiment.

Table I: Combination of different species of microalgae and artificial diet for rearing of *H. molitrix* (T_1-T_8) and *B. gonionotus* (t_1- t_8) fingerlings

Sl. No.	Treatments	*Scenedesmus obliques* (%)	*Spirulina platensis* (%)	*Chlorella ellipsoidea* (%)
1	T_1 & t_1	100	-	-
2	T_2 & t_2	-	100	-
3	T_3 & t_3	-	-	100
4	T_4 & t_4	33.33	33.33	33.33
5	T_5 & t_5	50	25	25
6	T_6 & t_6	25	50	25
7	T_7 & t_7	25	25	50
	T_8 & t_8			
8	(Control)	50% fishmeal and 50% mustard oil cake		

Fingerlings of *H. molitrix* (21 days old) were collected from local government (Maskanda) fish seed farm and *B. gonionotus* (35 days old) were collected from the nursery pond situated in the hatchery complex of Bangladesh Agricultural University (BAU) campus, Mymensingh. The fingerlings were reared in aquaria containing 30 L water in each, and the density of the fingerlings was one fish per 2.0 L of water. Before stocking in the experimental aquaria, acclimatization of fingerlings was done for a period of three days in 6 aquaria at a density of 70 fish per aquarium. In each step of experiment three replications were used both for control and microalgae feeding culture. The experiment was designed to fit to the CRD (Completely Randomized Design) (Gomez and Gomez 1976). The fingerlings were allowed to fed with cultured microalgae twice daily at the rate of 5% of body weight. Weight of cultured microalgae *Chlorella ellipsoidea* and *Scenedesmus obliquus* was calculated on the basis of OD_{620} =2.5- 3.1, 0.80 -0.95 g/1 dry biomass (Habib *et al*., 2003). On the other hand for determining the weight of cultured *Spirulina platensis* samples were filtered with filter paper (Whatman, GF/C) and shifted to the oven at 70^{o}C for 24 hours. The samples were then transferred to the dessicator for cooling and weight was measured using an electric balance. Before filtering the weight of the dried filter paper was taken. And the weight was calculated by the following formula:

Cell weight (mg/1) = Weight of the filter paper with sample - Weight of the filter paper without sample before filtering

Sufficient dissolved oxygen was ensured providing air pump in plastic tubing. Temperature, pH and dissolved oxygen (DO) of the water were determined every alternate day by respective meter. Weight (g) and length (cm) of fish fingerlings were determined once in a week by an electrical balance and a centimeter scale, respectively.

Estimation of Specific Growth Rate (SGR, %/day) of Fingerlings

Specific growth rate (SGR, %/day) of fingerlings were calculated by the following equation (Brown, 1957) :

$$\text{SGR (\%/day)}) \frac{\text{LnW}_2 - \text{LnW}_1}{T_2 - T_1} \text{X } 100$$

Where,

W_1 = the initial live body weight (g) at time T_1 day
W_2 = the initial live body weight (g) at time T_2 day

Analysis of Proximate Composition

Dried samples of reared fish were analyzed for proximate composition following the standard methods of Horwitz (1984) in the Fish Nutrition Laboratory of the Department of Aquaculture, BAU, Mymensingh.

Statistical Analysis

Mean and standard deviations were calculated. Then the data were analyzed through an one-way ANOVA test using SPSS followed by Duncan's Multiple Range Test (Zar, 1984).

Results and Discussion

In the case of rearing of *H. molitrix* (where the treatments are mentioned as T_1-T_8) the maximum weight gain (%) obtained in treatment T_2 (feeding 100% *S. platensis*) and the minimum was obtained in the control (T_8). feeding 50% fishmeal and 50% mustard oil cake. However, negative weight gain (-4.29%) was observed in control. Specific growth rate (SGR, %/day) were 1.97, 2.84, 1.60, 2.15, 1.28, 1.78, 1.33 and -0.21 in treatments T_1 - T_8, respectively (Table II). Significantly ($p<0.05$) higher survival rate (77.80%) was observed in T_2 than others while T_8 showed the least (25.90%). Negative growth indicates that *H. molitrix* didn't ingest supplied artificial diet. The SGR of the treatment T_2, feeding with 100% *S. platensis* was significantly ($p<0.05$) higher than other treatments. The maximum weight gain (g) was determined 0.56 in T_2 followed by 0.39, 0.35, 0.32 0.28, 0.23, 0.22 and -0.03 in T_4, T_1, T_6, T_3, T_7, T_5 and T_8, respectively (Fig. 2). The length gain showed the similar trend (Fig. 1). The survival rate was also the maximum in treatment T_2 and was significanlty ($p<0.05$) higher than other treatments (Table II). The findings of the present study regarding different growth parameters illustrate that *H. molitrix* fingerlings of treatment T_2 showed significantly ($p<0.05$) higher growth performance than other treatments. It may be happened due to the higher protein content of supplied *S. platensis*. Fish fry basically need high protein rich live foods for their development and because of that many researchers have been worked on the protein requirements of fish (Kaushik, 1992). But the overall growth performance of *H. molitrix* was not satisfactory. Live foods sometimes do not give good growth and survival rate of fish fry (Ozkizilcik and Chu, 1994).

During the rearing of *H. molitrix* fingerling, pH and dissolved oxygen (DO) ranges of water in the aquaria were 7.16 to 8.25 and 4.77 to 7.17 mg/1, respectively which were in suitable range. On the other hand during the study period the temperature range was 27.57 to 30.63^{o}C which was also within the suitable range of fish culture (Azim *et al.*, 1995 and Wahid *et al.*, 1997).

In case of rearing of *B. gonionotus* (where the treatments are mentioned as T_1 -T_8) the maximum weight gain 149. 10%

Table II: Growth performance and survival rate of reared *H. molitrix* fed on cultured microalgae in different combination

Treatments	Weight gain (g)	Weight gain %	Length gain (cm)	Length gain %	SGR (%/day)	Survival rate (%)
T_1	0.35 ± 0.04^{bc}	50.73 ± 0.03^{c}	0.61 ± 0.13^{b}	13.70 ± 0.08^{d}	1.97 ± 0.30^{b}	51.10 ± 5.85^{c}
T_2	0.56 ± 0.02^{a}	81.16 ± 0.03^{a}	0.80 ± 0.03^{a}	17.54 ± 0.03^{a}	2.84 ± 0.16^{a}	77.80 ± 5.54^{a}
T_3	0.28 ± 0.06^{cd}	40.00 ± 0.04^{e}	0.60 ± 0.13^{b}	13.48 ± 0.09^{e}	1.60 ± 0.43^{bc}	51.10 ± 3.85^{c}
T_4	0.39 ± 0.05^{b}	56.52 ± 0.04^{b}	0.73 ± 0.06^{ab}	16.30 ± 0.05^{b}	2.15 ± 0.39^{b}	55.60 ± 4.45^{c}
T_5	0.22 ± 0.06^{d}	30.56 ± 0.05^{g}	0.57 ± 0.16^{b}	12.70 ± 0.09^{f}	1.28 ± 0.34^{c}	53.30 ± 6.67^{c}
T_6	0.32 ± 0.06^{bc}	45.71 ± 0.05^{d}	0.66 ± 0.06^{b}	14.80 ± 0.05^{c}	1.78 ± 0.32^{bc}	64.40 ± 3.85^{b}
T_7	0.23 ± 0.09^{d}	32.86 ± 0.08^{f}	0.56 ± 0.04^{b}	12.56 ± 0.06^{f}	1.33 ± 0.14^{c}	53.30 ± 3.57^{c}
T_8	-0.03 ± 0.01^{e}	-4.29 ± 0.01^{h}	0.01 ± 0.01^{c}	0.22 ± 0.01^{g}	-0.21 ± 0.01^{d}	25.90 ± 5.54^{d}

Data in a column followed by different letter (s) indicate significant differences at 5% level

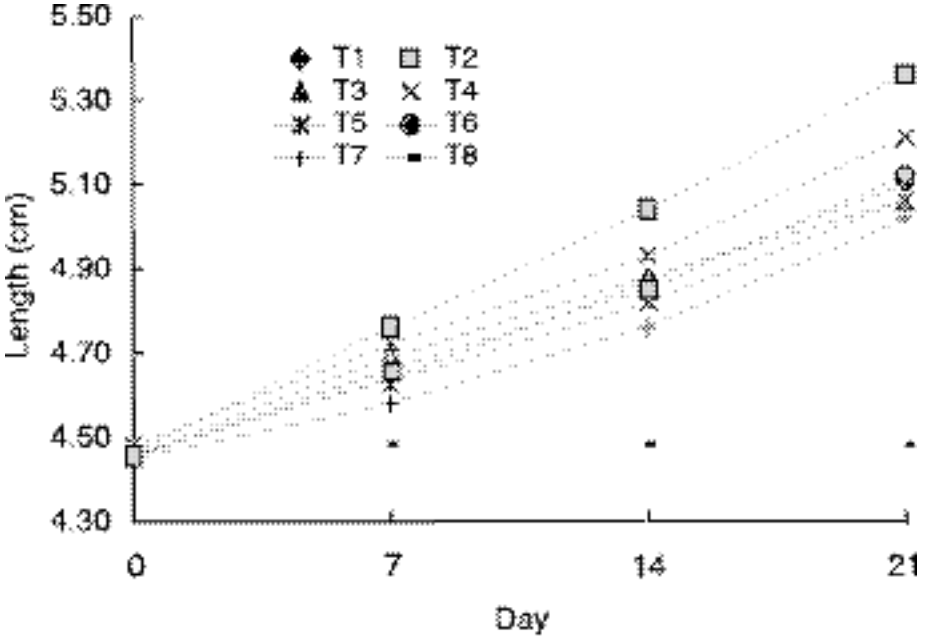


Fig. 1 Mean length (cm) of *H. molitrix* fingerlings under laboratory condition

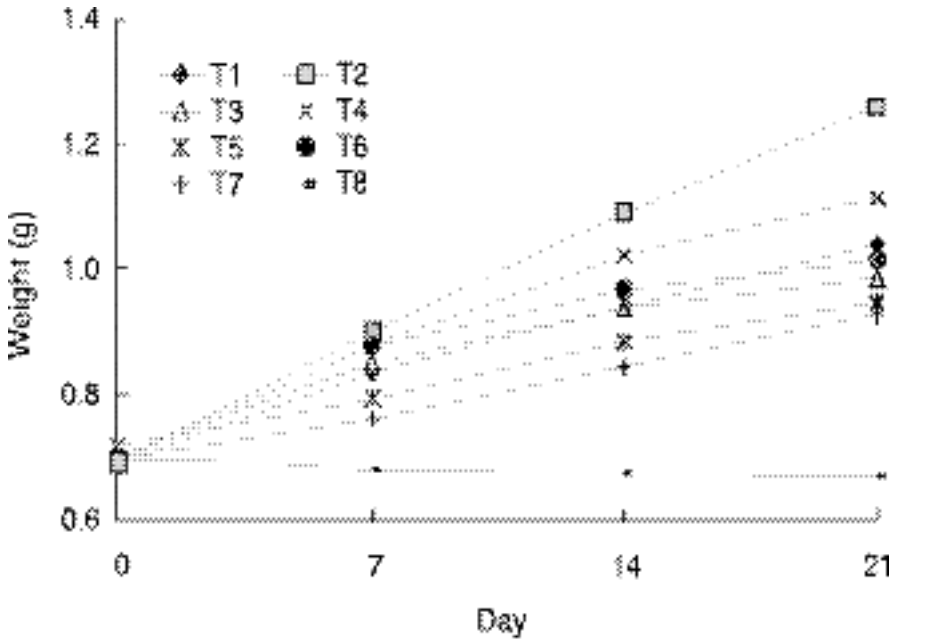


Fig. 2 Mean weight (g) of *H. molitrix* fingerlings under laboratory condition

was observed in t_2 followed by 124.2, 100.0, 73.68, 65.0, 65.0, 62.71 and 60.35 in t_8, t_5, t_3, t_1, t_4, t_6 and t_7, respectively (Table III). The length gain showed similar trend. In case of both weight gain and length gain treatment T_2 showed higher growth than other treatments (Fig. 3 and 4). Specific growth rate (SGR, %/day) was maximum (4.35) in the treatment T_2 feeding 100% *S. platensis* followed by 3.84, 3.30, 2.63, 2.44, 240, 2.32 and 2.25 in the treatments T_8, T_5, T_3, T_4, T_1, T_6 and T_7, respectively. Survival rate ranges from 97.78 to 100% for all the treatments and the differences was insignificant. However, no

Table III: Growth performance and survival rate of reared *B. gonionotus* fed on cultured microalgae in different combination

Treatments	Weirght gain (g)	Weight gain%	Length gain (cm)	Length gain %	SGR (%/ day)	Survival rate (%)
t_1	0.39±0.03^{d}	65.00±0.03^{e}	1.17±0.12^{c}	34.41±0.11^{e}	2.40±0.30^{d}	97.78+3.85^{a}
t_2	0.85±0.09^{a}	149.1±0.08^{a}	1.90±0.20^{a}	55.88±0.18^{a}	4.35±0.59^{a}	100.0±0.00^{a}
t_3	0.42±0.04^{d}	73.68±0.03^{d}	1.20±0.10^{cd}	35.50±0.10^{d}	2.63±0.36^{cd}	100.0±0.00^{a}
t_4	0.39±0.09^{d}	65.00±0.09^{e}	1.08±0.23^{cd}	31.86±0.22^{f}	2.44±0.59^{d}	97.78±3.85^{a}
t_5	0.60±0.08^{c}	100.0±0.07^{c}	1.50±0.15^{b}	44.12±0.14^{c}	3.30±0.50^{bc}	100.0±0.00^{a}
t_6	0.37±0.03^{d}	62.71±0.03^{f}	0.90±0.00^{d}	26.47±0.00^{g}	2.32±0.28^{d}	100.0±0.00^{a}
t_7	0.35+0.04^{d}	60.35±0.04^{f}	0.90±0.10^{e}	26.63±0.10^{g}	2.25±0.15^{d}	97.78±3.85^{a}
t_8	0.72±0.06^{b}	124.2±0.05^{b}	1.79±0.06^{a}	52.96±0.06^{b}	3.84±0.48^{ab}	100.0±0.00^{a}

Data in a column followed by different letter (s) indicate significant differences at 5% level

fish was died in the treatments t_2, t_3, t_5, t_6 and t_8. The different growth parameters of the present study illustrated that treatment t_2 (feeding 100% *S. platensis*) obtained significantly ($p<0.05$) higher growth performance than other treatments. It might be happened due to the higher content of protein and other nutrients of supplied *S. platensis*. The over all growth performance of *B. gonionotus* was considered satisfactory in the laboratory - based trial. Live foods sometimes give good growth and survival rate of fish fry (Craig *et al.*, 1994).

During the rearing of *B. gonionotus*, pH and dissolved oxygen (DO) of water in the aquaria were 7.28 to 7.89 and 4.13 to 6.40 mg/1, respectively and the temperature range was 28.27 to 29.47°C. These parameters were within the suitable range of fish culture (Azim *et al.*, 1995 and Wahid *et al.*, 1997).

The cultured microalgae contained 37 .21 to 58.38% protein, 11.15 to 17.37% lipid and 7.33 to 14.20% ash, 5.26 to 7.46% crude fiber, 7.62 to 9.73% moisture and 8.76 to 18.23%

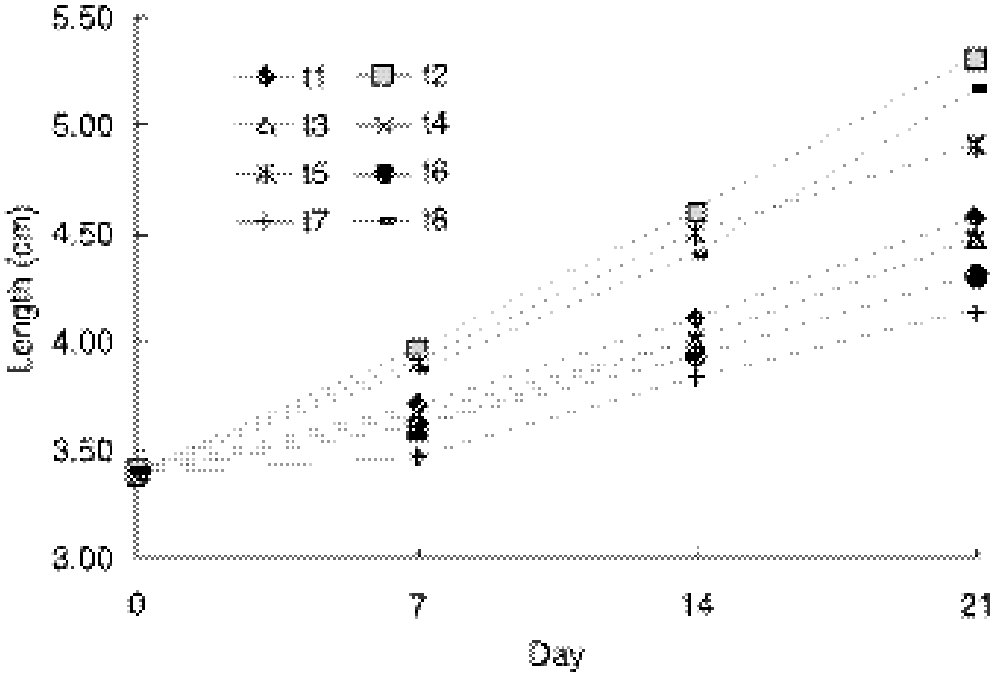
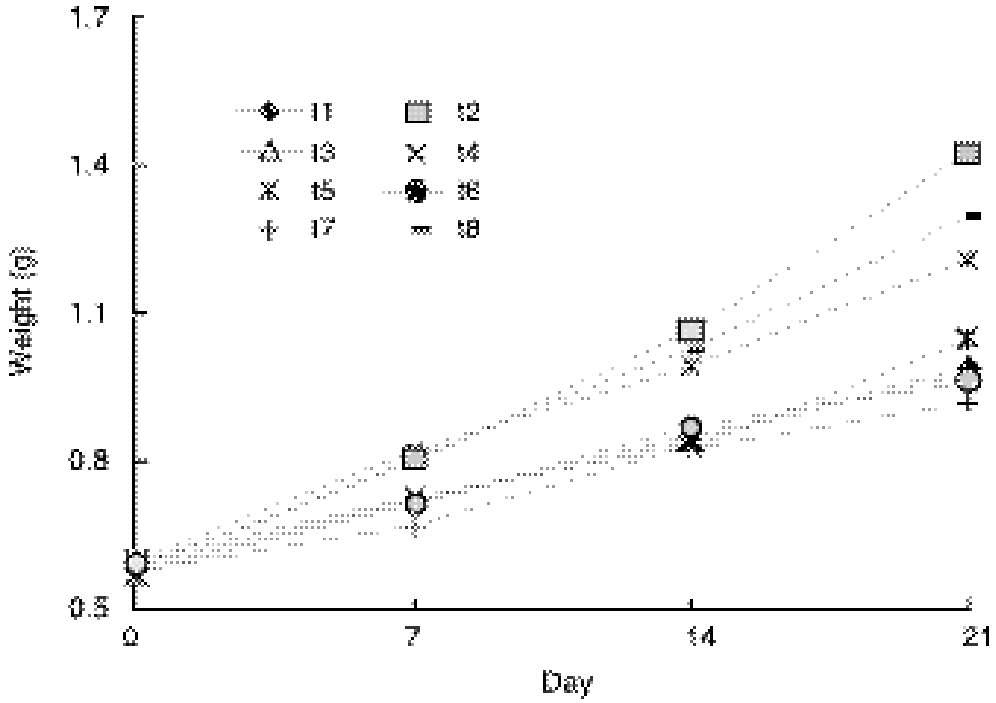


Fig. 3: Mean length (cm) of *B. gonionotus,* fingerlings under laboratory condition

Fig. 4: Mean weight (g) of *B. gonionotus,* fingerlings under laboratory condition



nitrogen free extract (NFE). In the case of *H. molitrix*, crude protein, crude lipid, ash and dry matter percentage were from 70.53 to 76.03%, 5.58 to 8.24%, 15.96 to 20.43% and 83.72 to 84.48%, respectively (Table IV). The maximum crude protein and ash percentage were observed in the treatments T_2, and T_4, respectively. Both crude lipid and dry matter percentage were maximum in T_5. In the case of *B. gonionotus*, the ranges of crude protein, crude lipid, ash and dry matter percentage were 73.62 to 77.05%, 5.38 to 6.71%, 16.87 to 18.50% and 82.97 to 84.32%, repectively (Table V). The maximum protein, ash and dry matter precentage of *B. gonionotus* were observed in the treatment T_2, but the maximum lipid was found in T_7.

The growth performance of *B. gonionotus* in all the treatments of the present study was comparatively better than *H. molitrix* The treatment feeding 100% *Spirulina* showed significantly ($p<0.05$) higher growth performance for both the

Table IV: Mean values (±SD) of proximate composition (% dry matter basis) of reared *H. molitrix* fed on cultured microalgae in different combination

	Composition (%)			
Treatment	Protein	Lipid	Ash	Dry matter
T_1	71.22±2.06^{ab}	7.03±0.31^{b}	19.94±0.69^{a}	84.10±0.26^{bc}
T_2	76.03±3.97^{a}	5.58±0.48^{e}	18.04±0.44^{b}	84.33±0.22^{ab}
T_3	73.86±1.35^{ab}	6.09±0.34^{de}	15.96±0.25^{d}	83.72±0.16^{d}
T_4	74.85±3.07^{ab}	6.38±0.28^{cd}	20.37±0.28^{a}	84.14±0.11^{abc}
T_5	74.54±4.17^{ab}	8.24±0.23^{a}	16.69±0.19^{c}	84.45±0.13^{a}
T_6	74.69±1.76^{ab}	5.91±0.19^{de}	18.62±0.66^{b}	84.13±0.10^{abc}
T_7	71.34±1.00^{ab}	6.26±0.19^{cd}	20.43±0.30^{a}	84.19±0.15^{abc}
T_8	70.53±3.11^{b}	6.73±0.21^{bc}	16.05±0.29^{d}	83.94±0.20^{cd}

Data in a column followed by different letter (s) indicate singnificant differences at 5% level

Table V: Mean values (± SD) of proximate composition (% dry matter basis) of reared *B. gonionotus* fed on cultured microalgae in different combination

Treatment	Composition (%)			
	Protein	Lipid	Ash	Dry matter
t_1	73.81±0.38^{d}	6.24±0.09^{bc}	17.49±0.67^{b}	83.63±0.46^{a}
t_2	76.75±0.78^{a}	5.38±0.11^{d}	18.50±0.22^{a}	84.32±1.09^{a}
t_3	75.47±0.21^{c}	6.40±0.29^{ab}	17.30±0.17^{b}	83.56±1.14^{a}
t_4	73.69±0.39^{d}	5.46±0.16^{d}	17.68±0.56^{ab}	83.88±0.60^{a}
t_5	75.08±0.84^{b}	6.63±0.19^{a}	16.92±0.58^{b}	82.97±0.43^{a}
t_6	74.61±0.32^{b}	5.98±0.18^{c}	17.51±0.74^{b}	83.59±0.72^{a}
t_7	73.62±0.46^{d}	6.71±0.21^{a}	16.93±0.47^{b}	83.80±0.74^{a}
t_8	77.05±1.54^{a}	5.42±0.12^{d}	16.87±0.53^{b}	83.78±0.75^{a}

Data in a column followed by different letter (s) indicate significant differences at 5% level

species of fingerlings which proved the suitability of *Spirulina* as a live food for fish. The species *H. molitrix* generally grows well in large water bodies. So, this species may not be suitable for trial in aquarium in the laboratory.

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References

Azim M. E., Talukder G. S., Wahab M. A., Haque M. M. and Haq M. S. (1995). Effects of liming and maintenance of total hardness levels on fish production in fertilized ponds. *Progress. Agric.* **6** (2): 7-14.

Bhanou K. K. and Vass K. K. (1973). A simple and inexpensive method for the mass culture of *Chlorella* sp. *J. Inland Fish. Soc. Indi.* **5**: 141-142.

Brown M. E. (1597). Experimental studies on growth. In: Brown, M. E. (ed.). The Physiology of Fishes. Vol. l. Academic Press, New York, USA. pp. 361-400.

Craig S. R., Arnold C. R. and Holt G. J. (1994). The effects of enriching live foods with highly unsaturated fatty acids on the growth and fatty acid composition of larval red drum, *Sciaenops ocellatus.J. World Aquaculture Soc.* **25**(3): 424-431.

De Silva S. S. and Davy C. (1992). Fish nutrition research for semi intensive culture system in Asia. *Asian Fish. Sci.* **5**: 129-144.

Gomez K. A. and Gomez A. A. (1976.) Statistical procedures for agricultural research (2nd ed.). Int. Rice Res. Ins., Philippines. 641 pp.

Habib M. A. B., Yusoff F. M., Phang S. M. and Mohamed S. (2003). Culture and nutritional value of *Moina micrura* Pallas fed on *Chlorella vulgaris* grown in digested palm oil mill effluent. *Asian Fish. Sci.* 1**6**(1-2): 107-119.

Horwitz W. (1984). Official Methods of the Analysis of the Association of Official Analytical Chemists (14th ed.). Association of Official Analytical Chemists; Washington D. C., USA. 1018 pp.

Jhons W. E. (1970). *Chlorella* for rearing fish larvae. *FAO Fish Culture Bull.*, **2**: 3pp.

Kaushik S. J. (1992). Fish nutrition science and aquaculture development. In: Aquaculture Research Needs for 2000AD. Oxford and IBH Publication, New Delhi, India. 414 pp.

Lovell T. (1991). Feed formulation and processing In: Lovell, T. (ed) Nutrition and feeding of fish. PP. Van Nostrand Reinhold Publication, New York, pp. 107-127.

Ozkizilcik S. and Chu F. E. (1994). Evaluation of omega-3 fatty acid enrichment of *Artemia* nauplii as food for striped bass *Morone saxatilis* Walbaum larvae. *J. World Aquaculture Soc.* **25**(1): 147-154.

Pruder G. D. (1983). Biological control of gas exchange in intensive aquatic production system. *J. Ins. Electro. Eng*. pp. 1002-1004.

Walne P. R. (1981). Culture of shellfish. In: Hawkins A. D. (ed) Aquarium System. Academic Press, pp. 347-371.

Wahid M. I., Haq M. S., Wahab M. A. and Ahmed Z. F. (1997). Effect of fertilizer treatments on the water quality and fish production in semi-intensively managed ponds. *Progress. Agric.* **8**(1&2): 61-65.

Watanabe T. C., Kitajima and Fujita S. (1983). Nutritional value of live organisms used in Japan for mass propagation of fish: *a review Aquaculture,* **34**: 115-143.

Yap Y. N., Wu J. F., Pond W. G. and Krook L. (1982). Feasibility of feeding *Spirulina maxima*, *Spirulina platensis* or *Chlorella* sp. to pigs weaned to a dry diet at 4 to 8 days of age. *Nutri. Reports Int.* **25**: 543-552.

Zar J. H. (1984). Biostatistics. prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA. 718 pp.

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