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Larval Rearing of Giant Gourami, *Osphronemus goramy* Lacépède 1801 Fed with Different Live Food Organisms

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Abstract

Assessment of growth performance, survival and body crude protein of giant gourami, *Osphronemus goramy* Lacépède 1801 larvae fed with different live food organisms was conducted to identify the most suitable live food for larval rearing. Growth performance (mean final weight and length) of giant gourami larvae was significantly highest in enriched *Moina* (55.3 ± 0.7 mg and 16.27 ± 0.29 mm), followed by unenriched *Moina* (41.0 ± 0.6 mg and 14.73 ± 0.17 mm), unenriched *Panagrellus redivivus* (Linnaeus 1767) (31.7 ± 0.3 mg and 10.47 ± 0.35 mm), unenriched *Artemia* (31.0 ± 0.3 mg and 12.20 ± 0.23 mm), enriched *Artemia* (31.0 ± 0.3 mg and 13.20 ± 0.61 mm), enriched *P. redivivus* (31.0 ± 0.3 mg and 10.53 ± 0.75 mm) and mixed-zooplankton (19.7 ± 0.9 mg and 8.87 ± 0.24 mm). Highest mean survival of giant gourami larvae was obtained in enriched *Moina* (96.95 ± 1.21 %) and the lowest mean survival rate was obtained in mixed-zooplankton (74.72 ± 1.47 %). Correlation analysis of crude protein content of live food organisms showed a positive linear effect on body crude protein content of giant gourami fry. Enriched *Moina* was the most suitable live food for the larval rearing of giant gourami based on growth performance and survival. The use of *Spirulina* powder, fish oil and baker's yeast was adequate for the enrichment of *Moina* for it gave a significant increase in crude protein content compared to other enriched live food organisms.

Keywords: larval rearing, live food organisms, enrichment, giant gourami

Introduction

Giant gourami *Osphronemus goramy* Lacépède 1801 is an important species in the ornamental industry. Being an edible species, it has also become one of the main species being cultured in Southeast Asia and is in great demand in the aquaculture industry (Azrita 2015).

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However, the main constraint in giant gourami culture is the source of fry such that most growers acquire their seeds from the wild. Therefore, commercial scale propagation of this fish in hatcheries is yet to be standardised. In aquaculture, the main objective of any hatchery system is to produce a maximum number of high-quality fish seeds, fry and fingerlings from the available broodstock (Marimuthu and Hanifa 2007). But, the success in the hatchery production of fish fingerlings for stocking in the grow-out production system is mainly dependent on the availability of suitable live food organisms for feeding fish larvae, fry and fingerlings to support good growth and health (Giri et al. 2002; Lim et al. 2003).

The possibility of replacing live feed with manufactured diets from the onset of exogenous feeding has been investigated in several studies (Jones et al. 1993, Person-Le Ruyet et al. 1993). However, information on the feeding of giant gourami larvae is needed for optimisation of large-scale culture and management of fish stocks. Based on the experience of Bureau of Fisheries and Aquatic Resources-National Freshwater Fisheries Technology Center (BFAR-NFFTC), the critical period of larval rearing begins at the time that yolk absorption is completed. If giant gourami larvae do not begin to eat during that period, then they become weak and eventually die (Amornsakun et al. 2002, 2004).

Live food organisms contain all nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids and fatty acids (New 1998), hence, are commonly known as "living capsules of nutrition". Among zooplankton, *Moina* is a commercially important cladoceran rich in nutrients making it an excellent live food for the good growth and development of fish and prawn larvae (Habib et al. 2003). *Moina* has been extensively utilised as live feed organism for feeding young fish in the industry (Martin et al. 2006). *Moina* can be easily mass cultivated under varying conditions including low oxygen and high ammonium content (Sarma et al. 2003), as these water fleas rapidly reproduce (Yamasaki and Uchiyama 2001) and rapidly grow on a range of food sources (Patil et al. 2010). *Artemia* nauplii are used extensively worldwide as live food for the larval stages of commercially important freshwater and marine fish species due both to convenience and to the nutritional value of *Artemia* (Kolkovski et al. 1997).

The microworm, *Panagrelus redivivus* (Linnaeus 1767), potential live food for fish and crustacean larvae, can be mass cultured in a wide range of media, which offers the opportunity to produce isotopically distinct live food (Brüggemann 2012). The study of Chea et al. (1985) shows that microworms were very suitable as a starter feed for feeding kissing gourami (*Helostoma temmincki* Cuvier 1829) larvae after week 2 under tropical conditions. This study was conducted to provide suitable live food organisms for the maximum growth and survival of giant gourami during the larval rearing period. Moreover, this study aims to improve the nutritional status of live food organisms through enrichment using various media.

Materials and Methods

Experimental set-up

The experiment conducted at the Freshwater Aquaculture Center, Wet Laboratory, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines wherein 21 rectangular aquaria measuring 49.50 cm × 25.40 cm × 27.80 cm were used in the study. Each aquarium was filled with 20 L water and equipped with aerators (air stone) to maintain the dissolved oxygen concentration of the water. The aquaria were covered by nets to prevent the fish from escaping and unwanted species. The experiment followed a complete randomised design (CRD) with seven treatments and three replicates each. The seven treatments are the types of live food organisms fed to giant gourami fry for 30 days in aquaria. Treatment 1 was mixed-zooplankton collected from the breeding pond of giant gourami which was composed of *Moina* sp., mosquito larvae and insect larvae. Treatment 2 was unenriched *Moina* sp.; treatment 3 was enriched *Moina* sp., treatment 4 was unenriched *P. redivivus*, treatment 5 enriched *P. redivivus*, treatment 6 was unenriched *Artemia* and treatment 7 was enriched *Artemia*.

Experimental diet enrichment formulation and preparation

Live food organisms (*Moina, Artemia, P. redivivus*) were used in the study due to their availability and easy culture method for mass production for giant gourami larvae. *Moina, Artemia* and *P. redivivus* were enriched with the same enrichment media and quantity such as *Spirulina* powder (DXN Pharma, Malaysia), fish oil (ATC Healthcare, Philippines) and baker's yeast (Redstar, USA). *Moina* and *Artemia* were enriched with 1.0 $g.L^{-1}$ baker's yeast, 0.25 $g.L^{-1}$ emulsified fish oil and 0.100 $g.L^{-1}$ of commercially available powders of *Spirulina* (Loh et al. 2012). Unenriched *Moina* and *Artemia* were introduced into 4 L container at a density of 1000 $pcs.L^{-1}$ for 12–24 h at room temperature (27.5 ± 1 ºC). Aeration was provided to maintain the oxygen level at >5 ppm. Enriched *P. redivivus* was mass produced in 100 cm^2 plastic containers filled with 70 g oatmeal, 1.0 g baker's yeast, 0.25 g emulsified fish oil and 100 mg of commercially available powders of *Spirulina* (Loh et al. 2012). Cultured stocks were kept humid by spraying water and supplemented weekly with 0.5 g baker's yeast (Delbare and Dhert 1996).

Test fish and feeding

There were 2,520 pcs of giant gourami larvae used in the study. After complete yolk sac absorption, the larvae were subjected to rearing experiment for 30 days. The larvae were randomly distributed in the 21 aquaria with a stocking density of 6 $pcs.L^{-1}$. Initial mean weight and length and total weight (bulk weight) of larvae at the start of the experiment were measured. The test larvae were fed *ad libitum* thrice a day at 0900 h, 1300 h and 1600 h. Feeding rate for all the diet types was not measured but given in excess (*supra libitum*).

Satiations were determined based on visual observation of acceptance and refusal of feed. The fish unconsumed feed and excrement from the bottom of the aquaria were siphoned with a plastic pipe daily.

Water quality monitoring

Water qualities such as dissolved oxygen (DO), temperature and pH were monitored every day (0900 h and 1500 h). Dissolved oxygen, temperature and pH concentrations were measured using water quality multiparameter (Hanna-HI98194, Japan). Aeration was provided in each aquarium using aerators. An approximately two-third portion of the water in each aquarium was changed daily before giving the first feed ration.

Growth and survival monitoring

Weight was measured using the electronic balance to the nearest 0.01 g and length was measured by a ruler. The behaviour of the fish larvae was observed during the experiment, especially during feeding. Dead giant gourami larvae were removed and counted every 24 h to estimate the survival rate. On the last day of the experiment, all remaining larvae were counted for the calculation of survival rate. Final weight and length from different treatments were recorded and subjected to statistical analysis. Survival rates were calculated by taking into account the remaining and discarded larvae.

Crude protein analysis

At the end of the experiment, all fish from seven treatments were sacrificed and used for final carcass composition analysis following the standard methods by the Association of Official Analytical Chemists (AOAC 1984). Samples were oven dried (AX60-Carbolite Gero, Germany) at the BFAR-NFFTC, Fish Nutrition Laboratory for 24 h. Crude protein analysis of different types of live food and giant gourami was conducted at the FAC-Fish Nutrition Laboratory using automatic Kjeldahl analyser (UDK-159, Velp Scientifica, New York).

Data analysis

After 30 days of the feeding trial, all survived fish were counted, weighed and measured for determination of culture performance: final weight (FW), final length (FL), and survival. The crude protein content of different types of live food organisms and body protein content of giant gourami were subjected to correlation analysis. All growth parameters were subjected to one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range to test significant difference among the means. Differences were considered significant at $P < 0.05$. Data were analysed using SPSS version 17.0 (SPSS Inc., 2008) for Windows. The data were presented as treatment means ± SD.

Results

Growth performance of giant gourami larvae

Growth performance [final weight (FW); final length (FL)] and survival of giant gourami larvae were recorded and calculated at the end of the experiment. Mean initial weight and length of giant gourami used in this study for all treatment were 9.3 mg and 8.0 mm, respectively. During the experimental period, no disease, abnormality or deformity was noticed in the experimental larvae. Fish fed T3 (enriched *Moina*) resulted in the highest mean final weight (FW) (55.3 ± 0.7 mg) which was significantly ($P < 0.05$) higher than means in all other treatments followed by T2 (unenriched *Moina*) (41.0 ± 0.6 mg), which was significantly ($P < 0.05$) higher than the mean FWs in the remaining other treatments (Fig. 1). Treatment 6 (unenriched *P. redivivus*) (31.7 ± 0.3 mg), T4 (unenriched *Artemia*) (31.0 ± 0.3 mg), T5 (enriched *Artemia*) (31.0 ± 0.3 mg) and T7 (enriched *P. redivivus*) (31.0 ± 0.3 mg) followed but their mean FWs were not significantly different from each other. Treatment 1 (mixed-zooplankton) (19.7 ± 0.9 mg) had significantly lowest mean FW compared to other treatment means.

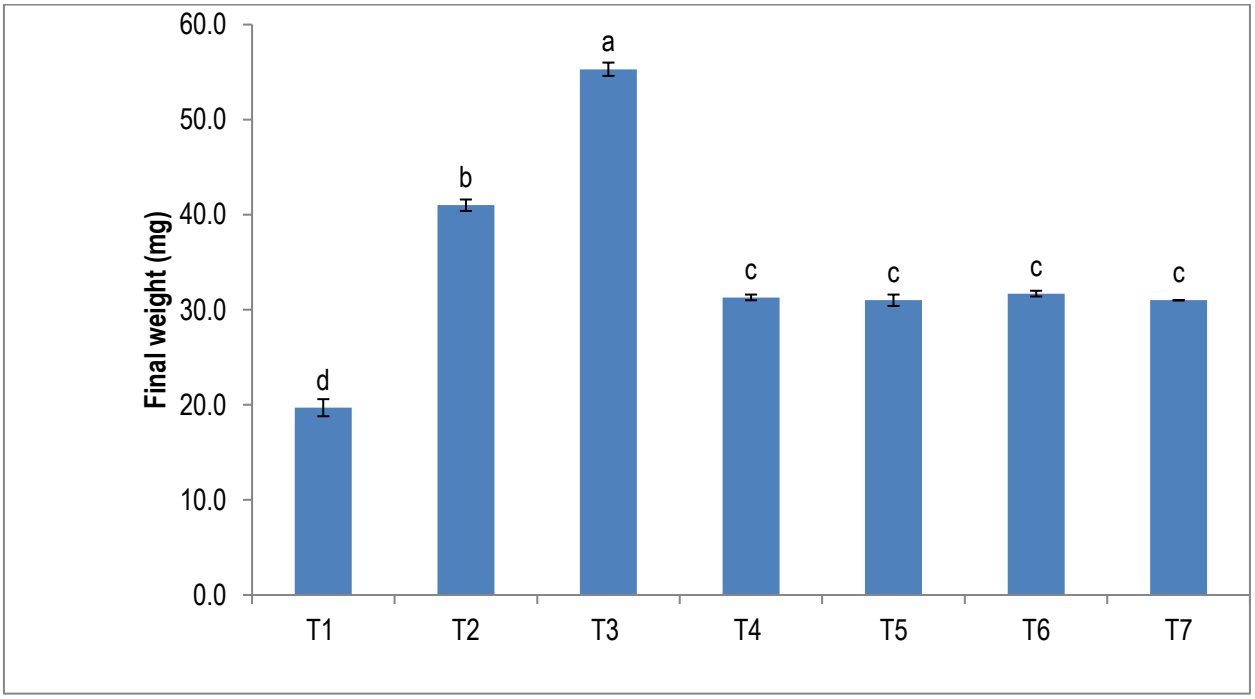

Fig. 1. Final weight of giant gourami after 30 days of feeding different live organisms. T1 mixed-zooplankton (composed of *Moina* sp., mosquito larvae and insect larvae), T2 unenriched *Moina* sp., T3 enriched *Moina* sp., T4 unenriched *P. redivivus*, T5 enriched *P. redivivus*, T6 enriched *Artemia* and T7 enriched *Artemia*.

Final length (FL) was highest in fish fed T3 (16.267 ± 0.291 mm) and was found significantly higher than in all other treatment means, followed by T2 (14.73 ± 0.17 mm), which was significantly ($P < 0.05$) higher than the mean FLs in the remaining other treatments (Fig. 2).

Mean FLs of T5 (13.20 ± 0.61 mm) and T4 (12.20 ± 0.23 mm) were not significantly different from each other. The same is the case between T7 (10.53 ± 0.75 mm) and T6 (10.47 ± 0.35 mm). Treatment 1 (8.87 ± 0.24 mm) had significantly lowest mean FL compared to other treatment means.

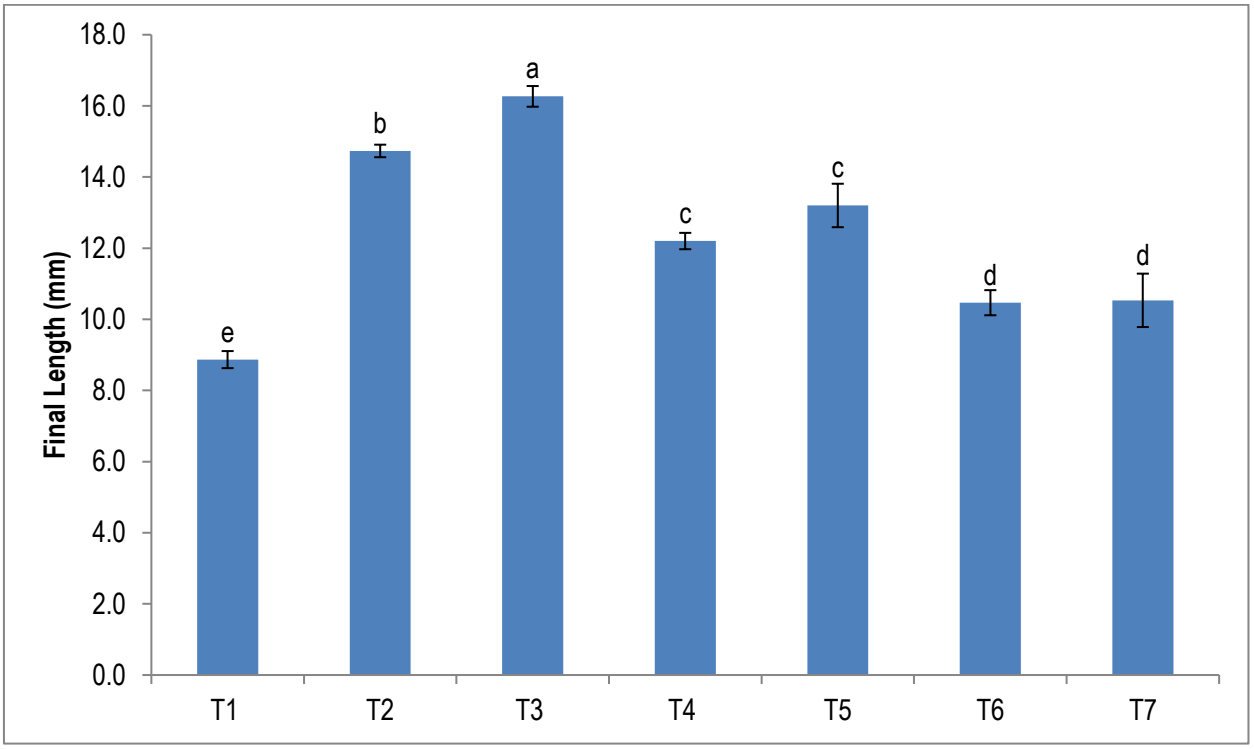

Fig. 2. Final length of giant gourami after 30 days of feeding different live organisms. T1 mixed-zooplankton (composed of *Moina* sp., mosquito larvae and insect larvae), T2 unenriched *Moina* sp., T3 enriched *Moina* sp., T4 unenriched *P. redivivus*, T5 enriched *P. redivivus*, T6 enriched *Artemia* and T7 enriched *Artemia*.

Highest mean survival rate was recorded in fish fed T3 (96.947 ± 1.21 %) but was not significantly different from mean survival rate of T2 (94.17 ± 0.96 %), T5 (87.78 ± 0.55 %) and T4 (87.22 ± 5.30 %). The lowest mean survival rate of the larvae was recorded in T1 (74.723 ± 1.470 %) but was not significantly different from those of T6 (77.78 ± 4.82 %) and T7 (83.33 ± 3.47 %) (Fig. 3). Water quality parameters such as dissolved oxygen (6.85–6.97 ppm), temperature (26.67–26.77 °C) and pH (6.67–6.73) were within the optimal range and were found to have no significant difference in all treatments.

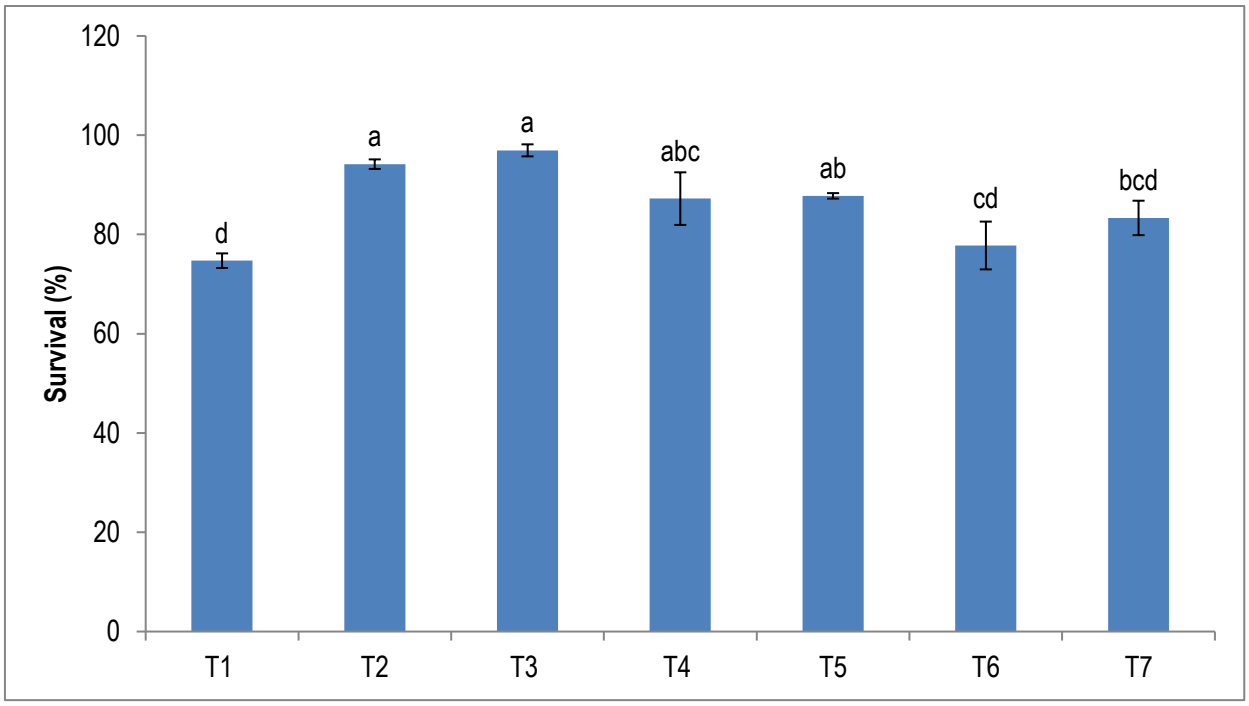

Fig. 3. Survival of giant gourami after 30 days of feeding different live organisms. T1 mixed-zooplankton (composed of *Moina* sp., mosquito larvae and insect larvae), T2 unenriched *Moina* sp., T3 enriched *Moina* sp., T4 unenriched *P. redivivus*, T5 enriched *P. redivivus*, T6 enriched *Artemia* and T7 enriched *Artemia.*

Crude protein composition of live food and body of giant gourami

The mean crude protein content of different types of live food organisms ranged from 40.42 ± 0.03 – 78.81 ± 0.04 %. All treatment means were found significantly different from each other. The highest mean crude protein content was obtained in fish fed T3 (enriched *Moina;* 78.81 ± 0.04 %) followed by T2 (unenriched *Moina;* 69.49 ± 0.51 %), T4 (unenriched *Artemia;* 64.14 ± 0.08 %), T5 (enriched *Artemia;* 63.41 ± 0.03 %), T7 (enriched microworm; 48.13 ± 0.06 %), T1 (mixed-zooplankton; 45.88 ± 0.03 %) and T6 (unenriched microworm; 40.42 ± 0.03 %). Statistical analysis showed there was a significant decrease of crude protein content in *Artemia* when subjected to enrichment process while the crude protein content of *Moina* and *P. redivivus* significantly increased.

Mean crude protein content of giant gourami fingerlings after 30 days rearing period ranged from 9.60 ± 0.18 - 18.29 ± 0.07 % (Table 1). The highest mean crude protein content of larvae was obtained in T3 (18.29 ± 0.07 %) and was found significantly different from other treatment. The lowest value of mean crude protein content was obtained in T1 (9.60 ± 0.10 %).

Results of correlation analysis showed that there was significant positive linear effect of the crude protein content of live foods organisms to body crude protein content of giant gourami (Fig. 4). As the crude protein content of live food organisms increases, the body crude protein content of giant gourami also increases.

Table 1. Protein analysis of different types of live food and body of giant gourami larvae after 30 days of larval rearing.

Treatment	Live food organisms Protein (%)	Giant gourami Protein (%)
T1 (Mixed-zooplankton)	$45.88^{f} \pm 0.03$	$9.60^{f} \pm 0.18$
T2 (*Moina*; unenriched)	$69.49^{b} \pm 0.51$	$17.16^{c} \pm 0.05$
T3 (*Moina*; enriched)	$78.81^{a} \pm 0.04$	$18.29^{a} \pm 0.07$
T4 (*Artemia*; unenriched)	$64.14^{c} \pm 0.08$	$17.42^{bc} \pm 0.03$
T5 (*Artemia*; enriched)	$63.41^{d} \pm 0.03$	$17.46^{b} \pm 0.09$
T6 (*P. redivivus*; unenriched)	$40.42^{g} \pm 0.03$	$14.90^{d} \pm 0.11$
T7 (*P. redivivus;* enriched)	$48.13^{e} \pm 0.06$	$14.53^{e} \pm 0.12$

Note: Means in a column superscripted by different letters are significantly different at 5 % level of significance.

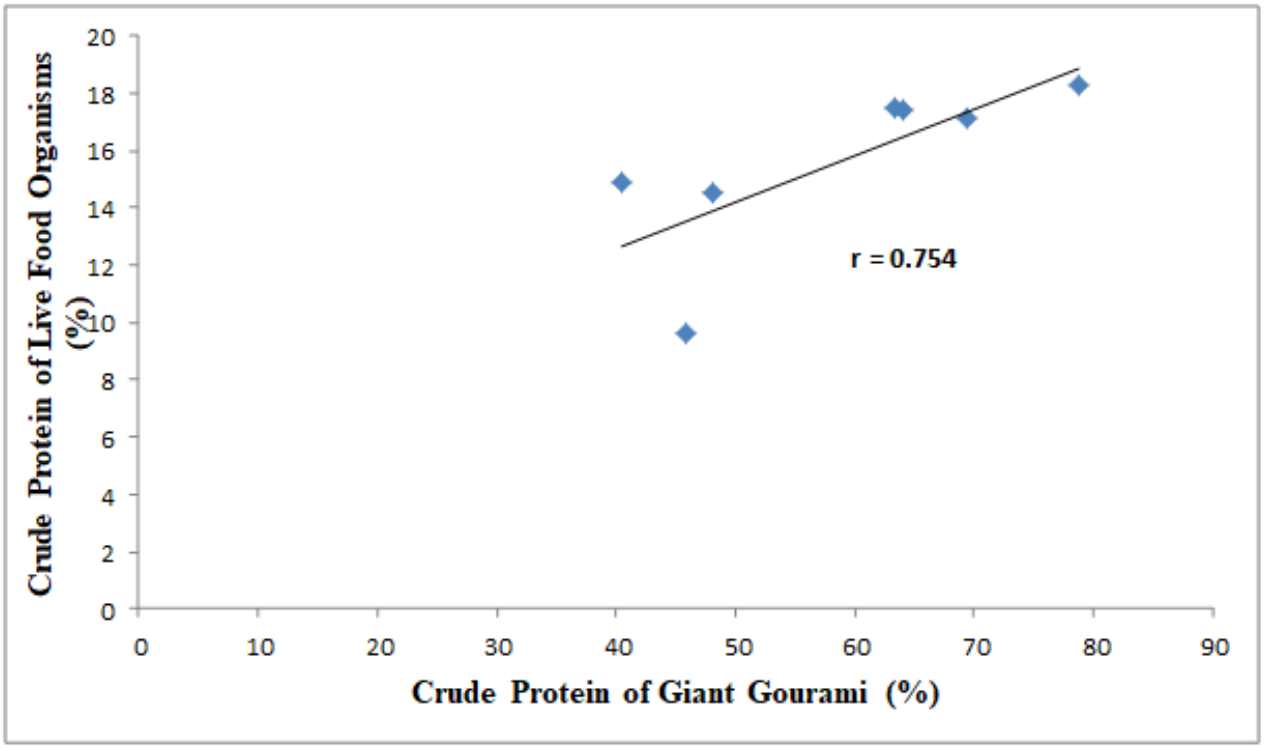

Fig. 4. Relationship between crude protein content of live food organisms and giant gourami larvae.

Discussion

Growth performance of giant gourami larvae

Live zooplankton contains enzymes (amylase, proteases, exonuclease, esterase) which play an important role in larval digestion (Munilla-Moran et al. 1990). Zooplankton was a better weaning diet of *Macquaria ambigua ambigua* (Richardson 1845) than *Artemia*. *Artemia* nauplii are nutritionally incomplete unless nutritionally boosted prior to use. Zooplankton harvested from ponds feed on algae and bacteria, which may provide nutrients absent in newly hatched *Artemia* (Herbert and Graham 2003). Generally, T3 (enriched *Moina*) gave the best performance than all other treatments regarding final weight, final length and survival and the highest protein (78.85 %) levels. Fermin (1991) found that *Moina* can be used as a partial or complete substitute for *Artemia*, provided *Moina* is sieved beforehand for efficient utilisation of the younger sea bass *Lates calcarifer* (Bloch 1790) larvae. The increase in the level of acceptance of giant gourami fed with *Moina* (unenriched or enriched) may be mainly due to its high protein content compared to other live food organisms.

Das et al. (2007) proved that growth rates of giant freshwater prawn *Macrobrachium rosenbergii* (de Man 1879) post larvae increased with increased amount of eicosapentaenoic acid and docosahexaenoic acid (HUFA-enriched) in dietary *Moina* compared to unenriched *Moina*. This is similar to the study of Indulkar et al. (2004) where postlarvae of giant freshwater prawn fed with different types of live food organisms. Results showed that *Moina* was superior to the other live foods with a significantly higher weight gain (88.86 ± 2.81 mg), length gain (20.71 ± 0.34 mm) and specific growth rate (9.45 ± 0.1 %.d^{-1}).

Adeyemo et al. (1994) studied the first feed for the fry of African catfish *Clarias gariepinus* (Burchell 1822) using *Moina dubia* Guerne and Richard 1892, mixed zooplankton (harvested from an earthen pond), *Artemia* nauplii and a commercial dry diet. After a 7-day fry nursing period, better growth and survival rates were observed for fry fed with *M. dubia* than for fry fed the other three diets. However, this is contrary to the study of Vartak and Singh (2009) where *Artemia* had better growth and survival than *Moina* when fed to sea bass fry. Moreover, Immanuel et al. (2001) revealed that survival of *Penaeus indicus* H. Milne Edwards 1837 fed with lipid enriched *Artemia* nauplii was higher than the control diet. Study of Kumar et al. (2008) showed that plankton groups (mixed) had lowest growth performance and survival when fed to striped murrel *Channa striata* (Bloch 1793) larvae.

Readings obtained neither significantly affected the survival nor the growth of the fish in each treatment. Boyd (1990) considered a concentration of dissolved oxygen above 5.0 mg.L^{-1} and temperatures of 20–28 °C are the desirable range for freshwater fish species. Swingle (1961) recommended pH variation between 6.5 and 9.0 to be the ideal range for most species of fish.

Crude protein composition of live food and body of giant gourami

Results of crude protein analysis for both unenriched and enriched microworms were similar to the findings of Rottman (1998) that microworms cultured in wheat flour are composed of 76 % water and 24 % dry matter; 40 % protein and 20 % is fat, while Lavens and Sorgeloos (1996) stated that the microworm contains 48 % protein, 21 % lipids, 7 % glycogen, 1 % organic acids, and 1 % nucleic acids. Moreover, Santiago et al. (2003) found that proximate composition of microworm using oatmeal was 38.8 % crude protein, 23.7 % crude fat and 28.9 % nitrogen-free extract. The use of enrichment methods affects the gross chemical composition of microworms. Schlechtriem et al. (2004) obtained microworms with different fatty acid compositions when the culture medium was enriched with sunflower oil or fish oil. Mixed zooplanktons collected from ponds were composed of cladocerans and insect larvae. The result of protein analysis of mixed-zooplankton in the present study showed lower value (45.88 %) than the findings of Kibria and Nugegoda (1999) that the zooplanktons collected from freshwater pond have 52.3–56.5 % crude protein. The low protein content of mixed-zooplankton in the present study could be the effect of low-density of phytoplankton present in the breeding pond of giant gourami, which serves as the primary food for zooplankton. According to Das et al. (2012), phytoplanktons or microalgae play an important role in aquaculture as means of enriching zooplankton for feeding fish and other larvae. In addition, microalgae provide protein (essential amino acids), energy and other key nutrients such as vitamins, essential polyunsaturated fatty acids (PUFA), pigments and sterols.

He et al. (2001) stated that the nutritional value of *Moina* is superior to commercially available newly hatched *Artemia* nauplii. Study of Loh et al. (2012) found that highly unsaturated fatty acids (HUFA) enhanced the essential lipid levels of *Moina macrocopa* (Straus 1820) through oil emulsions, e.g., squid oil, canola oil, and commercial products and these essential fatty acids promote the growth of other cladocerans (Müller-Navarra 1995). The result of protein analysis for both unenriched and enriched *Artemia* in the present study was closely similar to the findings of Zarei et al. (2006) where the crude protein of *Artemia* in dry matter basis was found to be 50.0–60.0 %. Enrichment of *Artemia* with *Spirulina* powder, fish oil and baker's yeast in the present study resulted in the decrease of the level of protein. Although enrichment increases the DHA:EPA ratio, the increase may be limited because the *Artemia* nauplii do not uniformly consume the enrichment and/or some of the ingested enrichment media are not metabolised before the nauplii are consumed by fish (Ohs et al. 2009). In addition, *Artemia* nauplii have the disadvantage of catabolizing DHA back to EPA.

Ahmad et al. (2012) reported that increase in the crude protein level increase carcass protein content of the fish. Govindan (1985) also demonstrated a range of 9–25 % protein content for freshwater and marine fish. Watanabe et al. (1983) reported an excellent protein efficiency ratio (PER) value of rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) fed with *Daphnia* and *Moina*.

Conclusion

The most suitable live food for the larval rearing of giant gourami for 30 days based on growth performance and survival was enriched *Moina*. Unenriched *Moina* can be considered as suitable live food for the larval rearing of giant gourami when it comes to survival as it ranked second highest to enriched *Moina*. The use of *Spirulina* powder, fish oil and baker's yeast is adequate for the enrichment of *Moina* for it gives a significant increase in protein content compared to other enriched live food organisms like *Artemia* and microworm. The use of *Spirulina* powder, fish oil and baker's yeast did not increase the protein content of *Artemia* during enrichment. Increasing or enriching the level of protein of the diet or live food organisms affects the level of body protein of giant gourami larvae. The level of body protein of giant gourami significantly increased as the level of the protein in live food increased.

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