Influence of Feed Types with Respect to Feeding Frequency on Larval Survival, Growth and Morphologyin the Siamese Fighting Fish, Betta Splendens (Regan)

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Abstract

B. splendens fry fed on *Infusoria* soup / rotation of feed showed the low mortality and higher growth parameters (MBW, MBL, SL and CFL) in the first week, *Infusoria* and *Artemia* nauplii (1 : 1 ratio) in the second week and *Artemia* nauplii alone in the third week. Duncan multiple range test revealed that, rotation of feed significantly ($P < 0.05$) enhanced high growth in fry as compared to other feeds. However, feed types did not influence the size of mouth in *B. splendens* fry. The length of air bladder was 2.5 times more in fry fed on rotation of feed as compared to fry received *Infusoria.* Fry received three meals a day significantly ($P < 0.05$) enhanced the tested growth parameters and length of air bladder (except head and mouth size) as compared to one or two or four meals a day. Fish fed with liver in all stages of *B. splendens* (fry, juveniles, adolescent and adult) elicited the higher feeding rate than those fed with *Artemia* and mixture diet; however, the trend was reversed in rate and efficiency of conversion. *B. splendens* fed with mixture diet in all stages elicited the high protein, lipid and energy contents in muscle and gonad tissues followed by fish fed with *Artemia* and liver.

Keywords

Feeding frequency, Morpometric, Air bladder, Mouth size,Feeding rate, *B. splendens,* Colour development, Rotation of feed

Introduction

Aquarium fishes accept a wide variety of live and formulated feeds. The success of ornamental fish culture depends mainly on the right choice of nutritionally balanced, non-polluting, economically viable and readily acceptable feed. The primary problem in rearing larval fish depends on size, quality and quantity of food (Abi-Ayad and Kestemont, 1994; James *et al.,* 1997).Supplementary feeding is a rout in practice in aquaculture to enhance the production of organisms to marketable size in a short period. Shortage of food supply reduces the survival, food intake and growth while excess feeding pollutes the environment and increases the production cost (Sampath, 1984).

Betta splendens is one of the most important ornamental fish species and its productions are limited due to the non-availability of suitable larval feed. The formulated diets are not found to enhance the larval survival and to stimulate the growth when used alone as a larval feed (Hogendoorn, 1980; Dabrowski, 1984; Prinsloo and Schoonbee, 1986). The reasons are that the larvae possess tiny mouth size and simple digestive system and hence unable to consume and digest formulated diets (Cahu and Zambonino Infante, 2001). Therefore, live feed organisms are considered as the right choice of feed at early larval stages. Moreover, natural live feed provides a substantial availability of easily digestible protein and essential nutrients (Jhingran, 1975; Ahmed, 1994; Nayak *et al.*, 2003b). During intensive larval rearing, the larvae may prefer live feeds like *Infusoria, Artemia* nauplii, rotifers etc. (Planas and Cunha, 1999). *Infusoria* is a live feed and its soup contains abundant of unicellular ciliates and flagellates, which are suitable as the first diet of *B. splendens* hatchlings and enhance the cent – percent survival and fast growth (James, 1998). Followed by feeding with *Artemia* nauplii and other planktonic organisms are essential. Besides, the growth of the hatchlings, fry and other stages are governed by number of meals. Many authors have studied the effect of nutrition on the growth of edible fishes (Kim *et al.,* 1996; James and Sampath, 2002a; Olurin and Oluwo, 2010). However, there is paucity of information on the effects of nutrition on the ornamental fishes (Lochmann and Philips, 1994; James and Sampath, 1998). Hence, the present chapter investigates the identification of suitable feed for hatchlings and number of meals and their effect on survival, growth, morphometric characters in *B. splendens.*

Materials and methods

Two series of experiments were conducted.

Series 1

Two hundred and forty individuals of 3 days old fry were collected from the laboratory bred brooders. They were sorted out into four groups. Each group comprised of 20 individuals was reared in circular plastic trough (10 *l* capacity) containing 5 *l* of water. Triplicates were maintained for each group. Fry were fed *ad libitum* with four different feed types. Group I was fed with *Infusoria*, Group II with *Artemia* nauplii and Group III with combination of both *Infusoria* and *Artemia* nauplii. Fry belongs to IV group was fed with *Infusoria* in the first week, combination of both *Infusoria* and *Artemia* nauplii in the second week and 3rd week by *Artemia* nauplii alone (rotation of feed). Fry were fed three times a day (6.00, 12.30 and 17.00 h). The experiment was conducted for a period of 21 days. Growth, morphometric measurements and survival were studied.

One hundred and eighty individuals of three days old fry were collected from laboratory bred brooders. They were sorted-out into four different groups in relation to four feeding frequency, one meal in a day (1/1), two meals a day (2/1), three meals a day (3/1) and four meals a day (4/1). Triplicates of each group consisted of 15 individuals were reared in circularplastic troughs (10 litres capacity) containing 3 *l* of water. Based on the result of 4.2.1., rotation of feed was chosen to study the impact of feeding frequencyon survival, growth and morphometric characters of fry.

Series 2

To study the growth in different life stages of *B. splendens,* four different developmental stages viz., fry, fingerlings, adolescent and adult were separately collected from the laboratory bred brooders. Each stage was divided into three feeding groups corresponding to three different types of feed namely *Artemia,* liver and mixture of both. Each group comprised of ten individuals was reared in circular cement aquaria (width: 58cm; height: 40cm; 120 *l* capacity) containing 50 *l* of water. *Artemia* sp. was collected daily from the salt pans. For feeding the fish, *Artemia* sp. was washed in freshwater three to five times to remove the salt from it. Beef liver procured from the local beef stall was kept in refrigerator. It was minced into small pieces and traces of water adhering to them, was removed by pressing between the folds of filter paper and was given to the 2nd feeding group. The 3rd feeding group wasfed by mixture of both.

Results

Larval survival

Fry fed on chosen four different feeds elicited the three distinct types of mortality. Fry received *Infusoria* soup elicited the low mortality (2%) upto 7 days and thereafter mortality was increased (35%) with time. Fry received rotation of feed and mixture showed early and low mortality of 2 and 10% respectively. However, the fry fed on *Artemia* nauplii elicited the mortality (21.65%) intermediate between *Infusoria* and mixture / rotation of feed groups (Table 1).

Larval growth and morphometric parameters

Feed types had a significant effect on the growth parameters of *Betta splendens* fry. Fry fed on *Infusoria* and rotation of feed elicited the maximum growth parameters (mean body weight, total body length, standard length and caudal fin length) (Fig. 4.2 – 4.4) in the first week. However, the selected growth parameters were high in fry fed on rotation of feed, followed by mixture diet, *Artemia* nauplii and *Infusoria* on day 21. Regression lines also showed the similar trends in the following order: rotation of feed > mixture > *Artemia* nauplii > *Infusoria* . Duncan multiple range test revealed that, rotation of feed significantly ($P < 0.05$) influenced the high growth in fry as compared to other feeds. The chosen growth parameters were also increased with an increase in rearing period and they elicited the better performance in the fry fed on rotation of feed. However, feed types did not influence the size of mouth in *B. splendens* fry (Table 2).The dorsal and anal fins rays were not developed in fry upto 7th

days in all feeding groups. However, the fins were developed into fold in nature, continuous fold, partially developed and completely differentiated and these morphometric characters were more pronounced in fish fed on rotation of feed, mixture, *Artemia* nauplii and *Infusoria* (Table 2. The number of caudal fin rays were increased with an increase in time in all tested feeding groups; however, they did not differ with one another. Fry fed on *Infusoria* was transparent and occurrence of chromatophore cells in head and trunk were low while color development was fast in fry fed on rotation of feed, mixture and *Artemia* nauplii. Air bladder began to develop on day 14 and it significantly ($P < 0.05$) increased with time in all groups. The length of air bladder in fry fed on *Infusoria* was 0.0.075 mm and it significantly increased to 0.123 ($t = 3.00$; $P < 0.05$), 0.185 ($t = 3.88$; $P < 0.01$) and 0.185 mm($t = 3.88$; $P < 0.01$) in fry fed on *Artemia* nauplii, mixture diet and rotation of feed respectively. There was 2.5 times more length of air bladder observed in fry fed on rotation of feed and mixture diet as compared to fry received *Infusoria* (Table 4.3).

Growth parameters

Feeding frequency influenced the growth of *B. splendens* fry in terms of mean body weight and length and caudal fin length. However, feeding frequency did not influence the growth of head and mouth size (Table 4.4). The mean body weight of fry fed on 3/1 Ff was 32.20 mg wet weight and it significantly declined to 28.93 ($t = 3.31$; $P < 0.05$) mg wet weight in fry fed with 4/1 Ff. Animals subjected to three meals a day had the highest total body length and standard body length followed by 4/1, 2/1 and 1/1 Ff . Duncan multiple range test revealed that, three meals a day enhanced the tested growth parameters (except head and mouth size) as compared to other feeding regimes. The differences in growth parameters in relation to feeding frequency and rearing period were statistically significant (ANOVA: $P < 0.01$;. The results obtained for air bladder length was similar to those of mean body weight; however, 3/1 and 4/1 Ff did not show any differences between them.

Developmental stages

Data presented in Tables 4.6 and 4.7 showed that feed types (*Artemia,* liver and mixture diet) significantly ($P < 0.01$) enhanced the feeding and growth parameters in all stages (fry, fingerlings, adolescent and adult) of *B. splendens* (Fig. 4.6 – 4.7). Fish fed with liver in all stages elicited the higher feeding rate than those fed with *Artemia* and mixture; however, the trend was reversed in rate and efficiency of conversion. For instance, the feeding rate of adult *B. splendens* fed with liver*, Artemia* sp. and mixture diet averaged to 46, 18, 34 mg g^{-1} fish day^{-1} and the conversion rate was 6.5, 8.0 and 13.5 mg g^{-1} live fish day^{-1} respectively. Similar trend was obtained in fry, fingerlings and adolescent also. *B. splendens* fed with mixture diet in all stages exhibited the moderate feed consumption, high growth rate with maximum conversion efficiency followed by fish fed with *Artemia* and liver. *B. splendens* fed with mixture diet in all stages elicited the high protein, lipid and energy contents in both tissues (muscle and gonad) followed by fish fed with *Artemia* and liver (except early stages). The quantum of protein, lipid, ash and energy content were increased with stages; however, an opposite trend was obtained in nitrogen free extract (Table 2; Fig. 1-2). Gonad began to develop in *B. splendens* when it attained the adolescent stage. Muscle exhibited the higher protein and energy contents than gonad in adolescent stage. In adult stage, the protein and lipid contents were high in gonad at pre – and post-spawning as compared to muscle. However, protein and lipids were maximum at pre-spawning and it significantly declined in both tissues at post-spawning. For instance, protein in muscle and gonad tissues was 49 and 65 mg g^{-1} wet weight respectively at pre-spawning and it correspondingly declined to 38 and 46 mg g^{-1} wet weight at post-spawning in fish fed with mixture diet. Similar results were also obtained in fish fed with *Artemia* and liver (Table 2). fish fed with mixture diet. Similar results were also obtained in fish fed with *Artemia* and liver (Table 2).

Table 1. Effect of feed types on survival of *Betta splendens* fry reared for 21 days. Twenty fry were reared in each feeding groups and each

value is the average performance of three observations.

Days	***Infusoria***			***Artemia* nauplii**			**Mixture**			**Rotation of feed (Rf)**		
	No. of fry dead/tested	**Per-cent mortality(%)**	**Per-cent survival**	**No. of fry dead/tested**	**Per-cent mortality (%)**	**Per-cent survival**	**No. of fry dead/tested**	**Per-cent mortality(%)**	**Per-cent survival**	**No. of fry dead/tested**	**Per-cent mortality(%)**	**Per-cent survival**
1	0/20	0.00	100.00	0/20	0.00	100.00	0/20	0.00	100	0/20	0.00	100.00
2	0/20	0.00	100.00	1/20	5.00	95.00	0.33/20	1.65	98.35	0.33/20	1.65	98.35
3	0.33/20	1.65	98.35	0.33/20	1.65	93.35	1/20	5.00	93.35			
4	0/20	0.00	98.35	1/20	5.0	88.35	0.33/20	1.65	91.70			
5	0/20	0.00	98.35	0.67/20	3.35	85.00	0.33/20	1.65	90.05			
6	0/20	0.00	98.35	1/20	5.0	80.00						
7	0/20	0.00	98.35	0.33/20	1.65	83.35						
8	0.33/20	1.65	96.70									
9	1.33/20	6.65	90.05									
10	1.00/20	5.00	85.05									
11	0.67/20	3.35	81.70									
12	1.00/20	5.00	73.35									

13	1.67/20	8.35	65.00									
14	0.33/20	1.65	63.35									
15	0.33/20	1.65	61.70									
16												
17 – 21												
Total	6.99/20	34.95	61.70	4.33/20	21.65	83.35	1.99/20	9.95	90.05	0.33/20	1.65	98.35

Table 2. Effect of feeds types on proximate compositions (mg g^{-1} wet weight) and energy (kJ g^{-1} dry weight) in muscle and gonad tissues of *Betta splendens* in relation to developmental stages. Each value is the average (X ∟ SD) of threeobservations

Developmental stages	Feed types	Muscle					Gonad				
		Protein	Lipid	Ash	Nitrogen free extract	Energy	Protein	Lipid	Ash	Nitrogen free extract	Energy
Fry	A	13.57±1.53	0.42±0.08	2.95±0.26	87.41±5.76	1.88±0.06	Gonad not developed				
	L	17.31±1.09	0.70±0.14	3.26±0.60	77.28±1.19	1.03±0.10					
	M	18.37±1.21	0.90±0.39	3.80±0.48	78.20±2.03	3.31±0.16					
Juveniles	A	18.83±0.39	1.74±0.03	4.56±0.40	75.06±0.31	3.37±0.10	Gonad not developed				
	L	21.97±0.66	1.70±0.03	3.48±0.40	73.00±0.45	2.89±0.06					
	M	22.78±0.11	2.43±0.02	3.04±0.15	71.70±0.24	4.46±0.23					
Adolescent	A	37.46±1.21	7.27±0.22	4.00±0.04	50.80±1.60	18.97±0.79	25.84±0.84	8.21±0.45	5.30±0.38	60.84±0.23	17.83±0.24
	L	29.25±0.21	7.00±0.02	3.20±0.09	60.45±0.25	18.10±0.53	22.72±0.22	5.97±0.05	4.48±0.15	64.07±1.62	16.32±0.28
	M	48.55±2.43	7.30±0.19	4.10±0.19	42.70±1.41	23.43±0.32	31.34±0.53	9.75±0.01	5.68±0.10	53.11±0.52	21.35±0.30
Adult Pre-spawn	A	39.68±4.36	13.60±0.04	4.55±0.11	46.95±1.10	26.87±0.17	55.66±2.09	15.85±0.01	6.32±0.03	22.17±2.13	26.53±0.70
	L	32.33±0.69	12.95±0.01	4.00±0.07	50.53±0.73	25.52±0.48	50.70±1.07	14.73±0.04	5.72±0.07	28.79±0.46	21.97±0.28
	M	49.37±1.50	14.75±0.18	4.96±0.44	31.27±1.91	27.24±0.11	65.35±0.82	12.36±0.06	7.43±0.07	9.86±0.65	27.20±0.16
Adult post-spawn	A	28.67±1.14	11.97±0.02	6.19±0.02	53.17±1.14	22.51±0.57	47.14±1.88	15.01±0.02	5.09±0.06	33.15±2.15	17.72±0.24
	L	24.63±1.62	10.95±0.04	5.18±0.23	59.24±1.38	20.40±0.31	31.84±2.12	13.00±0.24	4.28±0.18	50.03±2.26	17.01±0.20
	M	37.70±0.50	12.49±0.08	6.81±0.13	43.01±0.40	23.79±1.09	46.08±4.01	16.52±0.03	5.77±0.09	33.04±2.01	18.54±0.95

A – *Artemia* nauplii / *Artemia*; L – Liver; M – Mixture diet *Artemia* nauplii / *Artemia* and Liver

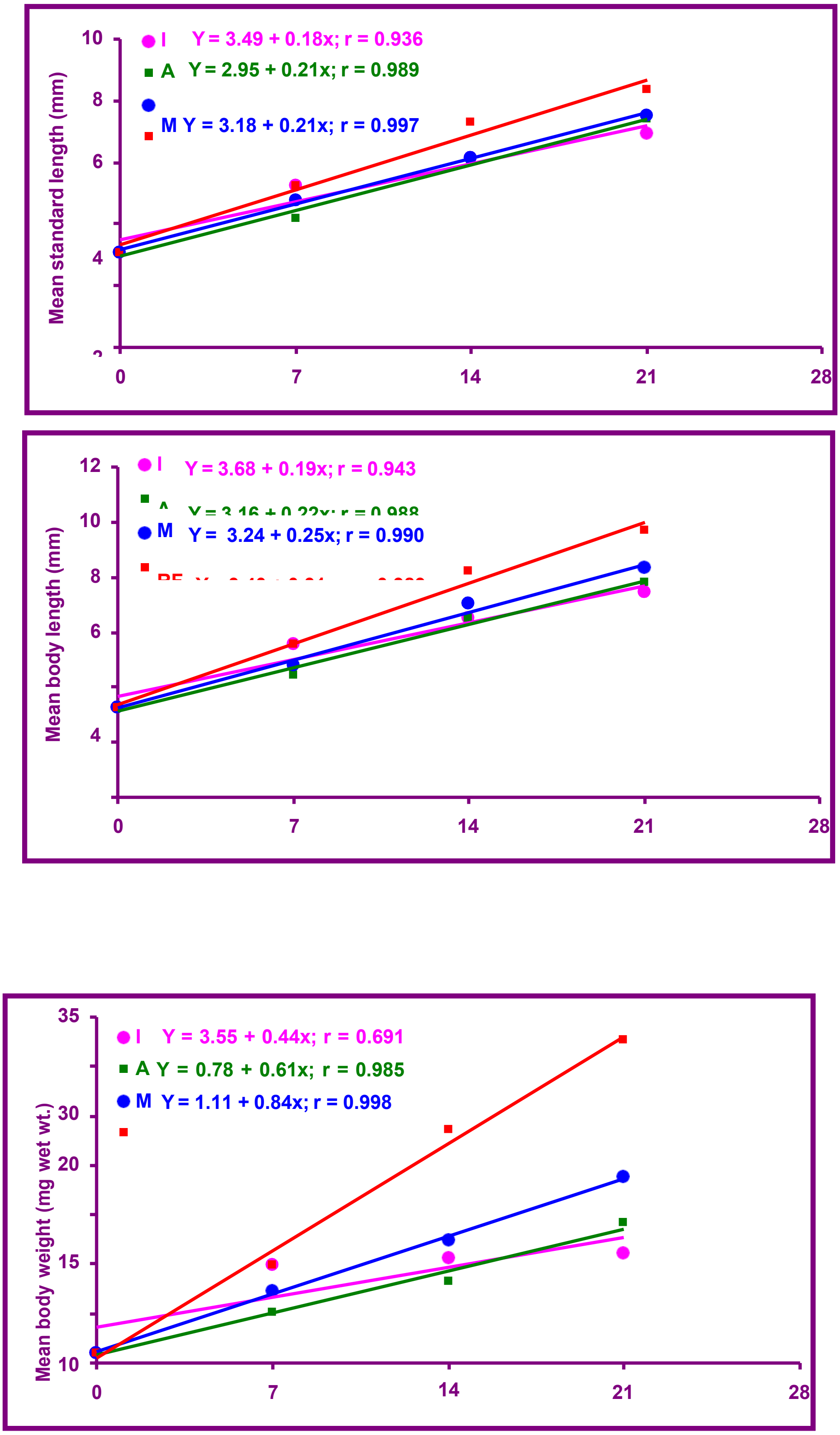

Fig. 1. Effect of feed types on mean body weight and length and

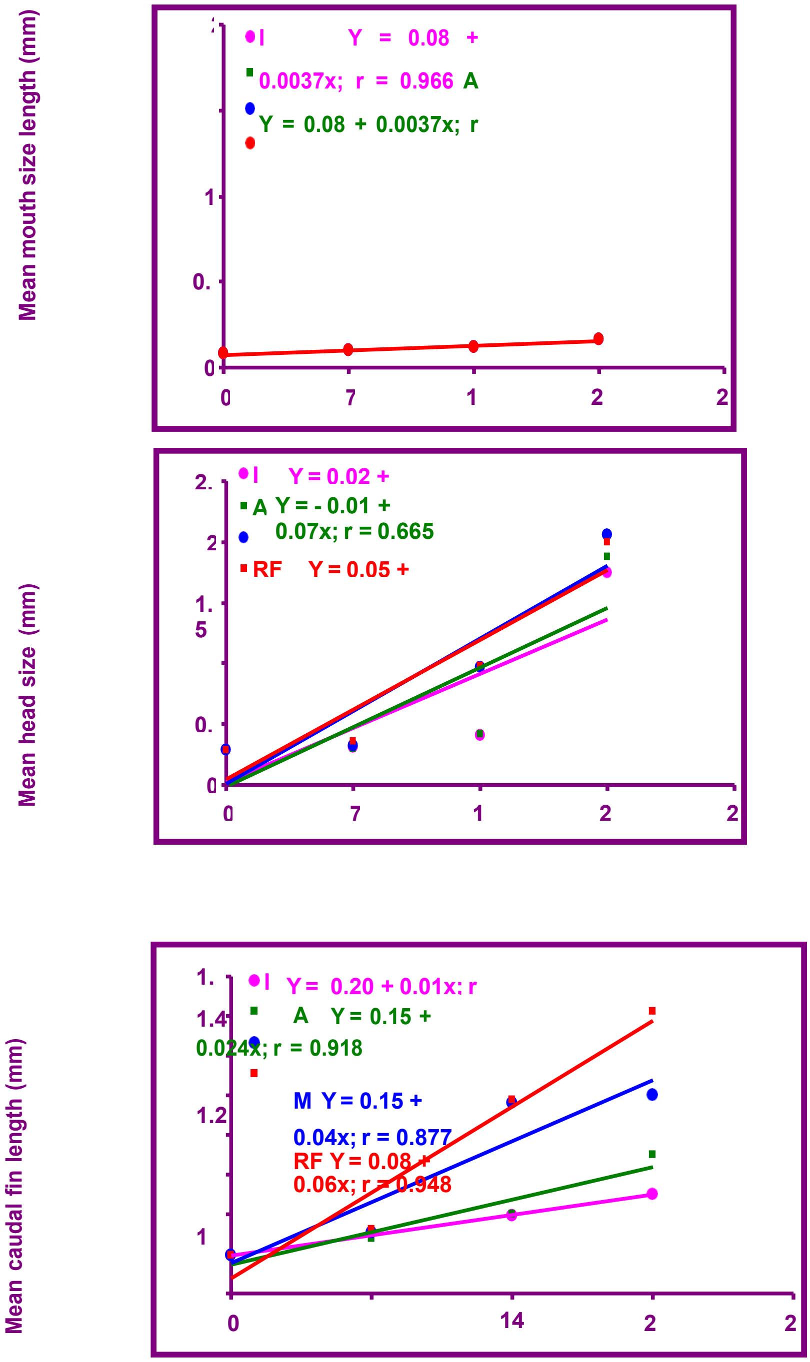

Fig. 2. Effect of feed types on mean caudal fin length, mean head size

Discussion

The present study revealed that *B. splendens* fry fed on *Infusoria* (upto 7 days of hatchlings / fry) showed a higher body weight, mean body length, mean standard length and mean caudal fin length as compared to fry fed with *Artemia* nauplii. This might be due to the small size of *Infusorians* (0.015 – 0.024 mm) to the suitable mouth size of fry (0.08 mm). Ogato and Kurolcura (2011) suggested that *Infusorians* are small sized first food, which are easily consumed by the small mouth size of fish larvae. The preference of *Infusorians* than *Artemia* nauplii was related to its size as well as prey type and the easy capture by inexperienced young predator (Stoecker and Govoni, 1984). The protozoa may provide equivalent nutrition like other live feeds if consumed by the fish larvae in large amounts (Howell – Kubler *et al.,* 1996). In addition, the poly unsaturated fatty acids (PUFA) necessary for the growth of the early fish larvae which may be taken from the ciliates in *Infusorians* soup (Rhoads and Kaneshiro, 1979). Less performance in the mixture diet upto 7 days may be attributed to the lack of sufficient *Infusorians* as feed to fry. Due to low density of *Infusorians*, the encounter rate will be infrequent and too much energy will be expended for searching prey resulted in poor growth (Davis and Dinis, 2002).

Fry fed on rotation of feed showed higher growth parameters (MBW, MBL, SL and CFL) since it was fed with *Infusorians* first week, *Infusorians* and *Artemia* nauplii (1:1) second week and *Artemia* nauplii third week. Aristazabal (2005) stressed the importance of shift the second live food item (*Artemia* nauplii) for feeding 13th day old *Pagrus pagrus* larvae. Sanchez-Velasco (1988) observed that *Callionymus* sp. and *Aenoglossus laterna* larvae preferred the changes in diet during their early ontogeny and further they also preferred larger prey organisms in their diet as the larvae grew. Moreover, enzymes such as amylase and trypsin found in *Artemia* nauplii (Samain *et al.,* 1980) and they promote the transition of fish larvae by enhancing the digestive efficiency (Leger *et al.,* 1986).

The present study showed that mouth development is slow and steady during experimental period. Morphological development of mouth was slow in *P. pagrus* larvae and it may be different with different species (Aristazabal, 2005). Fry developed coloration and the skin became opaque on day 21 in *Artemia* nauplii included in the feed types except *Infusoria* fed fry. The bright orange colour of *Artemia* nauplii was due to the presence of rich carotenoids in their body (Gilchrist and Green, 1960) and thereby *Artemia* nauplii fed fries showed colour development rather than *Infusoria* fed fries. However, no coloration observed upto 14 days even in *Artemia* fed fries suggests the inefficiency of fries to convert the available carotenoids. Ronnesstad *et al.* (1998) suggested that *Halibut* larvae were not able to convert the available carotenoids during the first phase of feeding, supports the present study.

The high survival in the fry fed on rotation of feed was due to the addition of *Infusorians* for the first phase and *Artemia* nauplii for the second phase from 14th day onwards. Lim and Wong (1997) observed higher survival in dwarf gourami fed with rotifer for the first phase (3 – 12 days) and *Artemia* nauplii for the second phase (13–32days) supports the present study. The fries mortality occurred on the first week in all feeds (except *Infusoria)* suggests it was a transition period from endogenous to exogenous feeding. The transition from endogenous to exogenous feeding is one of the most critical period in the development of fish larvae (Conides and Glamuzna,2001; Moteki *et al.,* 2001).

Feeding frequency had a significant effect on selected growth parameters. The optimum feeding frequency may vary with species and size of the fish (Goddard, 1996; Nadir Başçina *et al.,* 2007). Similar to our observation, 3 meals a day was found to promote higher growth in *Cirrihinus mrigala* fry (Sampath and Ravindran, 1988) and *Xiphophorus helleri* (James and Sampath, 2003a). It implies that this frequency of feeding was optimal for the enhancement of growth in *B. splendens* fry. Tsevis *et al.* (1992) reported that increasing feeding frequency than the optimum had resulted poor growth in sea bass. Moreover, more feeding (4/1 Ff) than the optimum level (3/1 Ff) led to food wastage and reduction in feed intake (James and Sampath, 2004c). The poor growth performance in 1/1 Ff was due to the lower food utilization and too inadequate for effective utilization (Priestley *et al.,* 2006).Higher feeding rate observed in liver fed fry, adolescent and adult *B. splendens* was due to the soft nature and palatability of liver (James and Sampath, 2002a). Although high feeding rate was observed in liver diet, the conversion rate was maximum in the mixed diet (*Artemia* and liver) fed fingerlings, adolescent and adult stage. This may be attributed to the higher pepsin activity contributed by exogenous supply from *Artemia* helps good assimilation and digestion. Most of the small fishes do not have sufficient enzymes for digesting non-living diets; exogenous enzymes from live food play an important role in assisting the digestive process in fish or crustacean larvae (Dabrowski, 1982; Jones *et al.,* 1993; Kamarudin *et al.,* 1999; Kolkovski *et al.,* 1997; Jafari *et al.,* 2011). In addition, *Artemia* nauplii may contribute to an activation of zymogens or digestive hormones (Petkam and Moodie, 2001) or may increase larval endogenous enzyme secretion (Pedersen and Hjelmeland, 1988) was another reason for the higher conversion rate in the mixed diet fed fishes. In the present study, low level of lipid in the somatic tissues and high level of lipid in the gonads indicates shifting of lipid from somatic tissues to gonadal tissues for the development of ovaries and mobilization of energy reserve to provide nutrients for the formation of eggs. Similar findings in other fishes support the present observation (Vetter *et al.,* 1983; Basade *et al.,* 2000; James and Sampath, 2004b). More organic reserves and energy contents in somatic and gonadal tissues in the adult at pre-spawn which declined drastically in adult at post-spawn indicates the utilization of stored organic reserves for the formation of eggs (James and Sampath, 2004b). Depletion of somatic energy noticed in the present study during spawning period may be due to utilization of energy for the act of courtship and spawning rather than gonad development (Dygert, 1990; James and Sampath, 2004b; Basade *et al.,*2000).

Conclusion

The present investigation suggests that, it is ideal to feed the fry by rotation of feed to enhance the survival and early growth. Rotation of feed is the suitable feed types and 3/1 feeding frequency is found to be the optimum meal frequency to maximize the larval survival and growth parameters in the hatchling of *B.* splendens so as to reduced the incidents of larval mass mortality and which always discourages the *Betta* rearers, Infusorians are the startthed starter feed in commercial *Betta* sreadrs to enhanced the survival of hatchlings in the first two weeks.

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