Evaluation of growth performance and breeding habits of fighting fish (*Betta splendens*) under 3 diets and shelters

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Abstract

There are various factors affecting the fighting fish larval survival including nutrition, substrate, water quality, pathogen and stress. Experiments were conducted to study the effect of substrate and live feed on larval survival of fighting fish fry. Nine small glass aquaria (60 cm × 30 cm × 30 cm) were used as breeding tanks. Equal pieces (80 cm^2) of rigifoam (T_1), polythene (T_2) and banana leaf (T_3) were placed on the tank surface to facilitate the male to form the bubble nest under it. Thirty days of feeding trial was conducted with three different live feeds (*Artemia*, *Moina* and *Bread worm*) as treatments with triplicates. The bubble nest area was significantly wider (p<0.05) under rigifoam (71.85±2.02 cm^2) and polythene (32.45±1.36 cm^2) than banana leaf (24.10±1.62 cm^2). Survival of fry was significantly higher under banana leaves (96%) and rigifoam (95%) than polythene (94%). Feeding trial, final lengths of fry were 1.65, 1.23 and 1.07cm for *Artemia*, *Bread worm* and *Moina* respectively whereas final weights were 0.05, 0.03 and 0.02g.Better survival rates were observed among treatments with no significant difference (p>0.05).Water quality parameters recorded in different treatments during the experimental period were pH (7-7.5), temperature (26-28 ^{0}C), and dissolved oxygen (5mg/L).

Keywords: *Betta splendens***,** Substrate, Live feed, Survival Introduction

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Introduction

Every year, ornamental fish demand increase worldwide. This clearly demonstrated by high rate of exports come from ornamental fish producing countries, which leads at present export market reached 11.6% in 2006 (Fishstat, 2011). Sri Lanka ornamental fish industry stared as commercial industry in 1952.Now it is a thriving industry with in export market, earning profit and employment opportunities for people (Kuruppan, 1998). Sri Lanka export fresh water, brackish water, marine water ornamental fish species and marine invertebrates. Fresh water ornamental fish species comprise more colorful, more attractive and striking fish species such as guppies, platy, mollies, swordtail, tetras, angels, gourami and fighter fish. Sri Lanka, Fighting fish is a highly demanded fresh water most beautiful, best-selling and popular ornamental fish species from the gourami family which is popular as an aquarium fish. The growth rate of Sri Lanka's aquarium fish industry is a healthy 4.7% with the share of 2.7% of the US$ 412 million world market.

Although successful breeding techniques have been developed for this species, high rate of mortality at the larval production stage is a critical issue. Fish larvae are usually very small, extremely fragile, and generally not physiologically fully developed (Laven and Sorgeloos, 1996). Combination of factors including nutrition, substrate, water quality, pathogen and stress decide the larval survival (Herath and Atapaththu, 2012).The breeding method of gourami family fish*(B. splendens)*is formation of bubble nest under floating substrate. Substrates are floating materials which are dominant in spawning area for construct the bubble nest.Bubble nests are aerated place for fertilized eggs in order to hatch those eggs successfully.

Quantity and quality of feeds are one of the other important affecting factors for growth performance and breeding of ornamental fish (Degani and Yehuda, 1996). In term of larval nutrition, many studies revealed that live feeds had a better growth and survival than formulated feed/Artificial feed. Within Live feeds, *Artemia* has extensivelybeen used in fighting fish larval rearing in Sri Lanka as the sole source of live feed since it ensures high growth and survival. But, high cost and occasional scarcity of *Artemia* makes it unsuitable for commercial aquaculture (Lim *et al*., 2003).

In recent years, there has been an increase in the interest of *B.splendens* in aquariums in Sri Lanka. High cost of the feeds and Selection of appropriate substrate for breeding were considered as the major constraint for fighting fish growers. There are scarcity of researches about selection of a suitable nutritionally balanced live diet (instead of *Artemia*) and substrate combination

in relation to growth performance and breeding of fighter fish. These above issues leads to provide suitable alternative feed for *Artemia* and substrate combination in Sri Lankan conditions become crucial for lowering the larval mortality and sustaining the production of ornamental fish especially of fighting fish.Therefore, this study is aimed to evaluate the effect of 3 different live feeds and substrate on the growth and breeding performance and breeding of Fighter fish in order to select suitable feed and substrate which is disease free, provide good complement of nutrients and gives better growth and breeding performance.

Review of literature

The Siamese fighting fish (*B. splendens* Regan, 1910), widely distributed throughout Southeast Asia, and is one of the most popular species of great commercial value for freshwater aquarium. *B. splendens* stands out as one of the most beautiful and best-selling ornamental fish from tropical water (Chapman *et al.,* 1997; Lima, 2003). In recent years, there has been an increase in the interest of *B.splendens* in aquariums in Sri Lanka. Moreover long-finned males are very important economically, providing the highest income among the exported ornamental fishes (Wiwatchaisaet, 2000).

Larval rearing is one of the key steps for any aquaculture achievement (Rinna Hamlin *et al*., 2014). But high rate of mortality at the larval production stage is a critical issue for fighter fish rearing. Special awareness should be taken for larval rearing because this stage is more pivotal than any other stages of life cycle (Rinna Hamlin *et al*., 2014). Fighter fish larvae are usually very small, extremely fragile, and generally not physiologically fully developed (Laven and Sorgeloos, 1996). Combination of factors including nutrition, Shelter, Water quality, Pathogens and stress decide the larval survival.

Fish that build and guard bubble nests are gourami (including *Betta* species) (Jaroensutasinee, 2005). Bubble nest building is the first step of the fish reproductive cycle (Hall, 1968). Bubble nests, also called foam nests, where males build bubble nests by blowing bubbles around a floating base. Females spawn under the nest, where the male waits to fertilize the eggs and typically 400-500 eggs are spawned by female fish (Sterba, 1983; Sakurai *et al*., 1992). Reproductive success of male fish mainly depends on size of bubble nest as it possible (Haung and Cheng, 2006). A larger bubble nest support more fertilized eggs and facilitates efficient oxygenation for those eggs and larvae (Jaroensutasinee, 2001).

In term of larval nutrition, many studies revealed that live feeds had a better growth and survival than that of larval fed dry feed. Most aquarium fish are unwilling to accept dried or flake foods therefore live food is occasionally necessary (Biokani *et al.,* 2014*).*

Artemia also represent the only practical food source for the early stages of many fish and crustacean larvae (Bardach, 1972). Siamese fighting fish is omnivorous as evidenced by aquarium specimens which will readily take most food provided. The quantity and quality of feeds are important factors affecting the Growth performance and reproduction in ornamental fish (Degani and Yehuda, 1996). So, proper live feed selection is important in early part of the growth stage of fish.

Materials and methods

Profile of the study area

The experiment was conducted in National aquatic resources research and development agency (NARA) in Rekawa Sri Lanka. All preparation needed for breeding of fighter fish was done in the laboratory. Aquarium set up, water supply facilities, working space were assured before the breeding program. The study period was January 2015 to April 2015.

Material and Instruments

Material: Fighter fish fingerling stock (Male and Female), breeding tanks, substrates for breeding, fighter fish feeds (live feed, formulated/commercial feed), aquatic plants, Oxygen Instruments: Electric balance, pH meter, Dissolved oxygen meter, Thermometer, Ruler, Oven, and all other necessary standard laboratory equipment were used as instrumentsemitter pump, oxygen stones

Methods

An experiment was conducted to study the effect of substrate on larval survival of fighter fish fry. Nine small glass aquaria (60 cm × 30 cm × 30 cm) were used as breeding tanks. One male fish was paired with female fish in each tank. Equal pieces ($80cm^{2)}$ of regiform, polyethene and banana leaf were placed on the tank water surface to facilitate the male to form the bubble nest under it. Different substrates such as regifoam, polyethene and banana leaf were considered as treatments T_1, T_2 and T_3. The bubble nest area was measured by ruler and number of fry Survive was counted. Water quality parameters recorded in different treatments during the experimental period.

The second experiment was thirty days feeding trial.That was conducted to evaluate the growth performance of larval fighting fish fed on three different live feeds (T1: *Artemia, T2: Moina*and T3: *Bread worm*) as treatments with triplicates. Five day old fry of fighting fish were stocked in glass aquaria (60 cm ×30 cm ×30cm) at a stocking density of 25 larvae /tank and fed on 3% of the body weight per day and sampled in every two weeks interval. Daily weight gain (DWG - mg day^{-1}), Specific growth rate (SGR %), Survivalrate (SR%) were computed.

Statistical analysis

All the statistical analysis was performed using the software SPSS (Version 16.0) and the significance of the difference between means was determined using Duncan's multiple range test. One-way analysis of variance was applied to determine the significant effect of different treatments on feeding parameters in *B. splendens*. The data were expressed by mean± standard deviation and differences were considered significant at $p<0.05$. In order to see the relationship among the parameters, scattered plot techniques were used.

Results

Water quality parameters recorded in different treatments during the experimental period were pH (7-7.5), temperature (26-28^{0}C), and dissolved oxygen (5mg/L).In aquariums with different color substrate, *B.splendens* prefers low light intensity for the construction of bubble nests. In this experiment, *B.splendens* has more significantly preferred to white color rigifoam than other color substrates, Moderate preference was observed under the green plant leaf. Lowest preference was observed for bubble nest construction under grey polyethene.

Moreover bubble nest area was significantly wider under the regifoam (71.85±2.02 cm^2) and polyethene (32.45±1.36 cm^2) treatments than that under the treatment with banana leaf (24.10±1.62 cm^2) (DMRT: $p<0.05$).Survival of fry was significantly higher under banana leaf (96%) and rigifoam (95%) than polyethene (94%). But larval survival of fighting fish did not significantly vary between the treatments of rigifoam, polyethene and banana leaf ($p>0.05$).

Larger healthier males form bubble nest continuously which facilitate to reduce daily decrease in size of bubble nest. But in the present study revealed, larger male don't construct large area bubble nest. There was weak positive (R=0.39) relationship between male body weight and construction of bubble nest area.

But In the present study of *B.splendens* was able to construct bubble nest in a wide temperature range of (25-29^{o}C). There was a strong positive correlation (R=0.88) was observed between the temperature and bubble nest area. The best temperature was 28^{o}C for bubble nest construction. According to the results, Regifoam was the better substrate than polythene and banana leaves in terms of bubble nest area and fry survival. Therefore it can be concluded that rigifoam can be used as a better substrate to facilitate good breeding performance and reduce the fry mortality of fighting fish.

Table 1: Bubble nest characteristics and survival rate of *Betta splendens* when allow for reproduction under three different substrates such as banana leaves, polythene and regifoam in glass tanks during experiment-01.

Each value is a mean ± SD of three replicate, Mean values with different superscript letters in the same row indicate significant difference at (p<0.05) (one way ANOVA and subsequently post hoc multiple comparison with DMRT).

Aspects	Parameters	Treatments		
		Banana leaves	Polythene	Regifoam
Bubble nest characteristics	Bubble nest area (cm2)	24.10±1.62^{c}	32.45±1.36^{b}	71.85±2.02^{a}
	Bubble nest diameter (mm)	2.17±0.57^{a}	1.17±0.29^{b}	2.83±0.58^{a}
	Bubble nest depth (mm)	4.17±0.58^{a}	2.83±0.58^{b}	2.83±0.57^{b}
Survival	Survival rate (%)	96.12±2.95^{a}	94.15±1.30^{a}	95.22±3.40^{a}

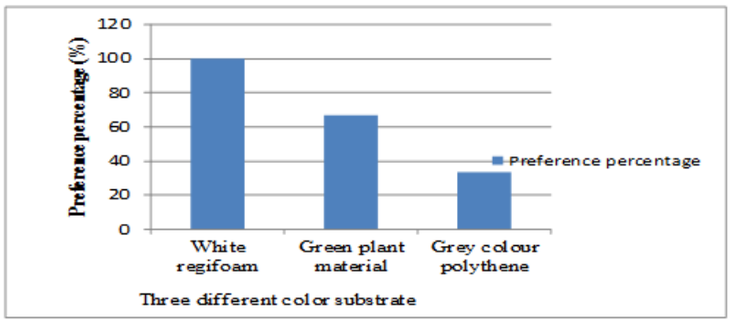

Figure 1: Different color substrate and preference percentage.

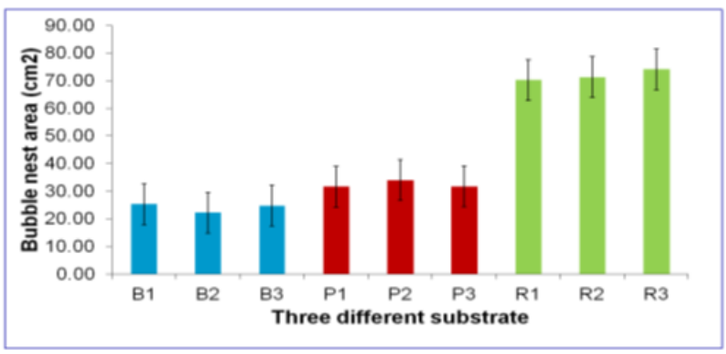

Figure 2: Different substrate and bubble nest area.

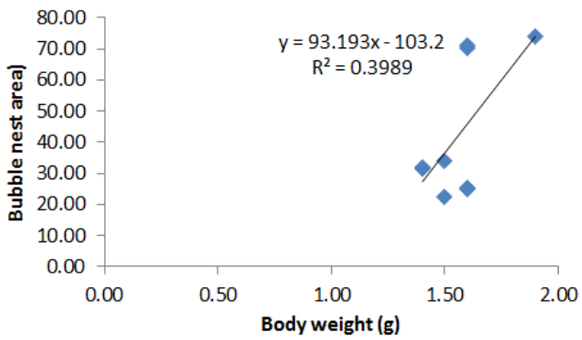

Figure 3: Correlation between body weight and bubble nest area.

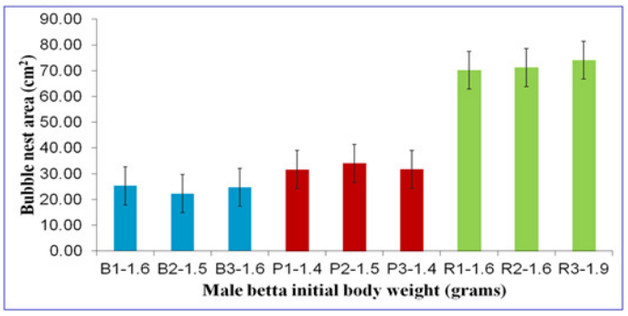

Figure 4: Male betta fish body weight and bubble nest area.

Experiment 02

Water quality parameters regarding feeding

pH for *Artemia*, *Moina* and Bread worm were 8.06±0.7, 7.91±0.13 and 7.89±0.12 respectively. Mean (±SD) values of 27.71±1.13 ^{0}C,27.61±1.05 ^{0}C and 27.64±0.72 ^{0}C were recorded for temperature and 7.00±1.02, 6.75±1.22 and 7.56±0.22 mg/L were recorded for DO during the period of experiment-2.

Final total length and weight was observed, *Artemia* fed group was significantly higher than other two treatments (p<0.05) (1.65±0.21cm, 0.05±0.01g). followed by *Bread worm* (1.23±0.17 cm, 0.03±0.00 g) and *Moina* (1.07±0.14 cm, 0.02±0.01g). There was significant difference observed in weight gain also; *Artemia* possessed a significantly higher WG followed by other two treatments. SGR was significantly higher for *Artemia* (3.71±0.27) lower for *Moina* (2.52± 0.31) and *Bread worm* (2.94± 0.00) has possessed a SGR in between *Artemia* and *Moina*. SGR observed for Artemia in the present study disagreed with the finding of Herath and Atapaththu (2012) in which *Artemia* fed fighter fish has shown SGR of 7.04±1.36 mg/day while Breadworm has shown higher SGR (2.94 mg/day) than the SGR observed for *Moina* (4.00±0.96) by Herath and Atapaththu (2012).

Table 2: Growth performance of B.splendens based on Initial and final total lengths, standard lengths and body weights, survival rate WG, SGR, FCR and FCE of B.splendens under three different live feeds such as Artemia, Moina and Bread worm in cement tanks during experiment-02.

Parameters	Treatments		
	Artemia	**Moina**	**Bread worm**
Initial total length (cm)	0.03±0.00	0.03±0.00	0.03±0.00
Final total length (cm)	1.65±0.21[a]	1.07±0.14[c]	1.23±0.17[b]
Initial standard length (cm)	0.25±0.00	0.25±0.00	0.25±0.00
Final standard length (cm)	1.42±0.01[a]	0.87±0.17[c]	1.05±0.15[b]
Initial weight (g)	0.0045±0.00	0.0045±0.00	0.0045±0.00
Final weight (g)	0.05±0.01[a]	0.02±0.01[c]	0.03±0.00[b]
Survival rate (%)	82.67±6.11[a]	80.00±0.17[a]	84.00±0.00[a]
WG (g)	0.045±0.01[a]	0.018±4.00[c]	0.025±0.00[b]
SGR (%)	3.71±0.27[a]	2.52±0.31[c]	2.94±0.00[b]
FCR	1.69±0.32[c]	4.19±0.92[a]	2.93±0.00[b]
FCE	0.61±0.11[a]	0.25±0.07[c]	0.34±0.00[b]

Each value is a mean ± SD of three replicate, Mean values with different superscript letters in the same row indicate significant difference at (p<0.05) (one way ANOVA and subsequently post hoc multiple comparison with DMRT).

Feed conversion ratio (FCR) and Feed conversion efficiency (FCE) was significantly higher for *Moina* (4.19±0.92) whereas FCE was significantly higher for *Artemia* (0.61±0.11). The FCR values observed in the present study agree with the findings of Mandel *et al.* (2010). In case of survival, there were no significant difference among 3 treatments. This evidence shows that any live feed among three of this live feeds can be used to obtain a good survival rate almost 80-84%.

Discussion

Water quality parameters such as pH, temperature, dissolved oxygen during the two experimental periods were more or less supported by the findings (Temperature: 26-28 ^{0}C, pH: 7-7.5, DO: 5mg/L) of Biokani *et al*. (2014).

Influence of temperature on reproductive behaviors

Temperature is one of the most potent environmental parameter that influences reproductive biology of fish (Herzig and Winkler, 1986). Which favor for maintaining its testes in a reproductively active. Initiation of bubble-nest building by the fighter fish indicates that the reproduction of the fish is commencing (Tsai, 1992; Huang *et al*., 1998). In our study seven male fish out of nine formed their bubble nest within 24 hours (77.78%). Temperature should be 25-28 ^{o}C for the reproduction of *B. splendens* in aquarium habitats (Reyes-Bustamante and Ortega-Salas, 1998).The present

study revealed that although *B.splendens* is able to construct bubble nest in a wide range of temperature (25-29^{o}C).The best temperature for bubble nest construction is 28^{o}C.

Influence of floating materials on reproductive behaviors

Floating plants are usually dominant in spawning areas of the fish (Jan, 1994). Plants or other substrates are necessary to hold on water surface (Degani, 1989). In aquarium habitats; *B. splendens* prefers lower intensity colors than dark color habitat for construction of bubble nest. Dark color was least preferred because they transmitted the least light. Bettas don't need that much of light for bubble nest construction (Harlioglu and Serpil Yonar, 2007). In the presence study betta male more preferred white color regifoam for construction of bubble nest. Subsequently green color leaf and gray polyethene were used as a substrate for construction of bubble nest.

Eggs floating at the water surface appeared more likely to develop than those submerged 10 cm below at the bottom of a glass jar. The behavior of male breeders could serve this physical function since males frequently express bubbles to the water surface, mouth the eggs, and place the eggs into the bubble mass. This sequence of nest-maintenance actions could increase the buoyancy and, indirectly, the survival of fertilized eggs. If the male fish want to achieve more reproductive success, it will maintain or enhance the size of the nest as much it can (Bronstein, 1982). In our study, the male fish was in good condition with white color regifoam they construct large bubble nest than other two substrates. But Survival not significantly vary between the treatments (p>0.05).

Effect of live feed on growth and performance of fighter fish fry and fry survival

The quantity and quality of feeds are important factors affecting the Growth performance and reproduction in ornamental fish (Degani and Yehuda, 1996). In term of larval nutrition, many studies revealed that live feeds had a better growth and survival than that of fry fed dry feed. In the fighter fish larval mortality reduction and enhance the growth rate at early stage and induce coloration these are induce by proper live feed selection. So, proper live feed selection is important in early part of the growth stage of fish.

Survival rate was significantly higher in *Artemia* subsequently to *bread worm* and *Moina*. Size of the prey and predator, nutritional quality, density, physical attractiveness and mode of presentation of food basically decide the feed intake of fish. Generally fish larvae are able to consume prey having same size of their mouth but small prey is more preferable. Live feed contain all the balance nutrition than artificial feed. Over the experimental period providing a wide spectrum of live feed and measured growth performance. Mouth gape of fish larvae decides the

preference of different size live feeds. Some finding revealed mixture of these three feeds also shows the better growth performance (Herath and Atapaththu, 2012). But correct ration mixture should needed. *Bread worms* are relatively smaller in size compare to *Artemia*. Hence, bread worms could be only suitable at the onset of larval feeding. *Moina* are comparatively larger than other live feeds and probably reduce the feed intake leading to the low growth and survival. The protein content of *Moina* usually averages 50% of the dry weight. Adults normally have a higher fat content than juveniles. The total amount of fat per dry weight is 20-27% for adult females and 4-6% for juveniles (Das *et al.,* 2012). *Artemia* cyst protein content 62% dry weight basis and 8% of crude fat (Hoseini**,** 2002).Bread worm contain 55% of protein on dry basis Fish might perform better if different size live feeds provide at different stages of larval oncogenic developments. Therefore larval rearing methods have to be improved further for commercial farming of the species. This experiment was done due to replacement of 100% *Artemia* by any other nutritionally equal live feeds. Morphometric parameters (Weight, Total length and standard length of fish) were significantly vary between treatments (p<0.05) and were significantly higher in *Artemia* compared to *bread worm* and *Moina.*

Weight gain (WG)

Final total length and Body weight was observed, *Artemia* fed group was significantly (p<0.05) higher (1.65±0.21cm, 0.05±0.01g) compare to other two feeds. Followed by *bread worm* (1.23±0.17 cm, 0.03±0.00 g) and *Moina* (1.07±0.14 cm, 0.02±0.01 g).

The result disagrees with WGs observed for *Artemia*, *bread worms*, *copepods* and mixture (Herath and Atapaththu, 2012).

Specific growth rate (SGR)

SGR was significantly higher for *Artemia* (3.71±0.27) lower for *Moina* (2.52± 0.31)and bread worm (2.94± 0.00) has possessed a SGR in between *Artemia and Moina.*

SGR observed for *Artema* in the present study disagreed with the finding of Herath and Atapaththu (2012) in which *Artemia* fed fighting fish shown SGR of 7.04±1.36 mg/day while *bread worm* shown SGR (2.94 mg/day).

Survival

There were no significant difference in survival between 3 treatments has provided evidence that any live feed among three of this live feeds can be used to obtained a good survival rate.

Feed conversion ratio (FCR)andFeed conversion efficiency (FCE)

FCR was significantly higher for *Moina* (4.19±0.92) whereas FCE was significantly higher for *Artemia* (0.61±0.11). The FCR values observed

in the present study in agreement with the findings of Mandel *et al*. (2010).

Conclusions

The present study was designed to evaluate the Growth Performance and Breeding of Fighter Fish under three feeds and substrates. The conclusion provided suitable feed and substrate combination that gives better growth and breeding performance. From our Study, regifoam treatment is the best substrate treatment than other two treatments. The bubble nest area was significantly higher under the regifoam (71.85±2.02cm^2) and polyethene (32.45±1.36 cm^2)substrates than banana leaf substrate (24.10±1.62 cm^2). Larger bubble nest areas can handle a greater number of fertilized eggs, provide more efficient oxygenation and support faster embryo development. Therefore, larger bubble nest males are able to achieve greater reproductive success.

So regifoam selected as best substrate for male preference for bubble nest building and fertilize more eggs. From the second experiment instead of the *Artemia, Moina and Bread worm* can be used as a best source of live feed during the larval stage of the fighting fish.

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