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Original Article

Effects of culture conditions on growth and survival of *Poecilia sphenops* and *Poecilia reticulata*

Sithara Rasanjalee Sirimanna*, Chamari Dissanayake

Department of Zoology, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.

Abstract: *Poecilia sphenops* and *P. reticulata* are considered as two most popular and high demanding freshwater ornamental fish species globally. The effects of feeding frequency, feed protein level, photoperiod and salinity on growth and survival of these species and the effect of sex ratio on fecundity were studied under laboratory conditions from January to December 2016. Although there was no any significant impact of feeding frequency on growth and survival of these species, feed protein content affected significantly on their growth. Fry fed with newly hatched *Artemia*, commercial larval feeds and *Chlorella* sp. showed no significant impact on growth. *Poecilia sphenops* and *P. reticulata* could tolerate salinity up to 10 and 25 ppt, respectively. Fish exposed to 8 hrs photoperiod reported significantly lower growth than those who were exposed to 12 and 24 hrs photoperiods. The ideal female: male sex ratio for commercial stocking of *P. sphenops* and *P. reticulata* was 3:2.

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Introduction

Ornamental fish culture is one of the high income generating practices in the world. Millions of fresh, brackish and salt water fishes belonging to 2,000 species worth around 250-400 million US\$ are annually traded as ornamental fish throughout the world (FAO, 2015). The United States is the single largest importer of ornamental fish in the world (FAO, 2015; Dey, 2016) while Singapore, Hong Kong, Malaysia, Thailand, the Philippines and Sri Lanka are considered as the top exporters. Favorable climatic conditions, natural water resources and ever growing market demand are some driving forces that promote the ornamental fish industry in Sri Lanka. Around 35 ornamental fish species have been exported from Sri Lanka in 2016 reporting an average income of 13 million US\$ (EDB, 2016). Both Guppy (*Poecilia reticulata*) and Molly (*P. sphenops*) belonging to the family Poeciliidae contribute more than 75% of ornamental fish exports from Sri Lanka (EDB, 2016; Perera, 2016).

Similar to other aquaculture practices, feed management, health management and water quality

management are the major concerns in ornamental fish culture. Feed is the most potent exogenous factor that affects growth and other physiological mechanisms in fish. Supplementary feeding is typically practiced in aquaculture to enhance growth of organisms to marketable size within a short period whereas limited feeding negatively affects survival, food intake and growth. Ration size and feeding frequency varies with the age, season and species of fish (Johnston et al., 2003). Excess feeding not only pollutes the water but also increases production costs (James and Sampath, 2003). Nutrient requirements of fish differ with the age and species but protein is considered as the most important nutrient in fish growth (Chong et al., 2000). Levels beyond the optimum protein requirement result in excess deamination and catabolism of protein and the lower protein levels result poor growth (Kruger et al., 2000). Environmental factors such as temperature, photoperiod, salinity and Dissolved Oxygen (DO) have a significant impact on growth, survival and reproduction of fish (Boeuf and Le Bail, 1999). According to previous literature, 23-27°C temperature

*Correspondence: Sithara Rasanjalee Sirimanna
E-mail: sitha9234tcc@gmail.com

(Dzikowski et al., 2001), DO level higher than 6 mg/l⁻¹ (Parameshwaran et al., 2001), Photoperiod of 12 hours (Boeuf and Le Bail, 1999) and 0 ppm salinity (Rosen and Bailey, 1963) are considered ideal for the optimum growth of freshwater ornamental fishes.

Considering all these factors, this study was carried out to determine the effects of feeding frequency, feed protein level, photoperiod and salinity on growth and survival of two highly exported freshwater ornamental fish species, *P. sphenops* and *P. reticulata*. An attempt was also made to identify the relationship between sex ratio and fecundity of these species. As ornamental fish industry is rapidly growing in the country with the support of government, this study would be a support to ornamental fish farmers in Sri Lanka to identify the most appropriate breeding and culture conditions for *P. sphenops* and *P. reticulata* to maximize the export revenue while enhancing the production and livelihood opportunities.

Materials and Methods

Brood stock collection and maintenance: Thirty brooders from each species, *P. sphenops* (1.2±0.2 g) and *P. reticulata* (1.0±0.2 g) were collected, from a commercial fish farm, packed in double layered polythene bags and immediately transported to the Aquatic science laboratory of the Department of Zoology, University of Sri Jayewardenepura. Upon arrival to the laboratory, brooders were quarantined with 2 ppt KMnO₄ for 30 minutes and transferred to the brood stock rearing tanks (glass tank with 55×30×30 cm) where the stocking density was 1 fish per liter. Brood stock rearing tanks were well-aerated and brooders were fed with commercial fish feed (PRIMA fish feed, produced by PRIMA Ceylon Pvt Ltd, Sri Lanka) twice a day based on 10% of their body weight (Harpaz et al., 2005). The bottom of each tank was siphoned out daily and the water quality parameters were maintained at pH, 7.2±0.2; temperature, 29±1 °C; dissolved oxygen, 6.0±0.3 mg/l; Ammonia, 0 ppm.

Experimental setup: The common experimental setup (Fig. 1) was used to study the effects of different culture conditions, i.e. feeding frequency, feed protein level, salinity and photoperiod on growth and survival

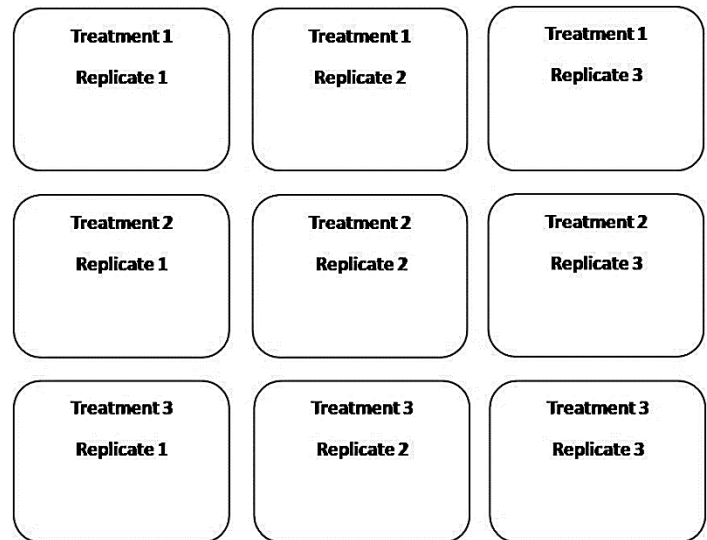


Figure 1. Common experimental setup used to study the effects of culture conditions on growth and survival of *Poecilia reticulata* and *P. sphenops* under laboratory conditions.

of *P. sphenops* and *P. reticulata* under the laboratory conditions. Each experiment was replicated thrice and 30 laboratory bred fry (2 day old) were used in each replicate tank. Initial length of fry both in terms of total length and standard length was measured before introducing them to the experimental tanks. Experimental fish were fed with commercial fish feed pellets (PRIMA fish feed) twice a day except for the feed frequency experiment. The tanks were provided with continuous aeration throughout the experimental period, tank bottoms were siphoned out daily and the water quality parameters were monitored twice a week. Fish were reared under natural day light condition except for those who were subjected to the photoperiod experiment and the water quality parameters in experimental tanks were maintained at pH, 7.0±0.2; temperature, 29°C±1; dissolved oxygen, 6.2±0.3 mg/l; Ammonia, 0 ppm. In all growth experiments, the standard length (cm) and wet weight (g) of each experimental fish was measured weekly. Length measurements were taken since the beginning of all experiments but the weight measurements were taken 8 weeks after the experiments as the individual weight of each fry was difficult to measure accurately.

Effect of feeding frequency: *Poecilia sphenops* and *P. reticulata* fish were fed with commercial fish feed (PRIMA fish feed) subjecting them into three different

Table 1. Main ingredients used to prepare experimental feeds with three different protein levels.

Ingredients	Diets		
	20% Protein	40% Protein	60% Protein
Danish fish meal (70%) ¹	125	125	125
Soya	16.3	126	235
Gelatin	15	15	15
Cod liver oil	15	15	15
Wheat flour	296.5	189	78
Vitamin and Mineral Mix ¹	25	25	25
Carboxy Methyl Cellulose	7.5	7.5	7.5
<i>Proximate Composition</i>			
Crude protein	20.5	39.3	59.3
Crude lipid	9.5	9.4	9.5
Ash (%)	4.3	4.5	4.3
Fibre (%)	4.3	4.6	3.9
Gross Energy (kJ/g) ³	16.5	16.5	16.5

¹Source: (Moisture 42.3 g kg⁻¹, Crude protein 706.80 g kg⁻¹, Crude lipid 74.70 g kg⁻¹, ash 92.50 g kg⁻¹ and fibre 1.60 g kg⁻¹).

²PREMIX; As per Kg, Vitamin A 2.0×10⁶ IU, Vitamin D3 4.0×10⁵ IU, Vitamin E 5.0 g, Vitamin C 20 g, Vitamin B1 0.8 g, Vitamin B2 1.0 g, Vitamin B6 2.4 g, Vitamin B12 40 mg, L-Lysine 3 g, DL Methionine 2 g, Choline Chloride 5 g, Niacinamide 10 g, Magnesium Sulphate 24 g, CobaltSulphate 80 mg, Sodium Selenate 20 mg, Pottassium Iodide 240 mg, Biotin 150 mg, Ferrous Sulphate 28 g, Copper Sulphate 24 g, Zinc Sulphate 24 g, Manganese Sulphate 6.8 g, Inositol 5 g, Calcium Carbonate as carrier.

³Gross Energy: Calculated based on 0.17, 0.40 and 0.24 kJ/g for carbohydrate, lipid and protein, respectively.

feed frequency treatments; once (8.30 a.m), twice (8.30 a.m and 4.30 p.m) and thrice (8.30 a.m, 12.30 p.m and 4.30 p.m) a day. The amount of feed given at a time was assigned based on 10% of mean body weight of experimental fish. This experiment was carried out for 32 weeks and growth and survival of each species were estimated with respect to three different treatments.

Effect of feed protein content: Three fish feed types each containing 20, 40 and 60% of protein were prepared following the method described by Chong et al. (2000) (Table 1) under the laboratory conditions and *P. sphenops* and *P. reticulata* were fed with experimental feed twice a day (8.30 a.m and 4.30 p.m) for a period of 24 weeks. The water Ammonia level was measured weekly throughout the experimental period and at the end of the experiments, growth and survival of two species were estimated with respect to three different feed types. Micro-Kjeldahl method with acid digestion was used to determine the crude protein content in prepared fish feeds and conversion factor 6.25 was used to convert total nitrogen to crude protein (Haider et al., 2015).

Effect of larval feeds: Effect of three different larval feed types; newly hatched *Artemia* nauplii, *Chlorella* sp. and commercial larval feed (PRIMA powder fish feed produced by PRIMA Ceylon Pvt Ltd, Sri Lanka) on the growth and survival of *P. sphenops* and *P. reticulata* fry was compared for a period of 4 weeks. *Artemia* nauplii were hatched in 32 ppt saline water and the *Artemia* concentration was maintained at 2 nauplii ml⁻¹ under laboratory conditions as described by Tolga (2012). Mass culture of *Chlorella* sp. was prepared using a stock solution and the green water (*Chlorella* sp.) with 1.5×10⁶ cells/ml cell concentration was maintained and daily cell counts were taken using Sedgewick rafter counting chambers (Raehanah et al., 2009).

Effect of salinity: Three days old fry (n=20) and six months old adults (n=20) of *P. sphenops* and *P. reticulata* were exposed to six different salinity treatments; 5, 10, 15, 20, 25 and 30 ppt. At the beginning of this experiment, all the experimental fish were kept in freshwater (0 ppt) for two days and then they were directly subjected to each saline treatment (Shikano and Fujio, 1997). The survival of

Table 2. Growth performance of *Poecilia reticulata* and *P. sphenops* in Feeding frequency, feed protein content and photoperiod experiments.

		<i>P. reticulata</i>						
		Mean Initial length (cm)	Mean Final length (cm)	LG (%)	Mean Initial weight (g)	Mean Final weight (g)	BWG (%)	SGR (%day ⁻¹)
Feeding frequency	1	0.6±0.0	2.2 ±0.3	72.7	0.2 ±0.0	0.7 ±0.1	71.4	0.54
	2	0.6±0.0	2.7±0.3	77.7	0.2 ±0.1	1.0 ±0.1	80	0.56
	3	0.6±0.0	2.7±0.2	77.7	0.2 ±0.0	1.2 ±0.1	83.3	0.81
Feed protein content	20%	0.6±0.0	1.4±0.1	57.1	0.1±0.0	0.4±0.1	75	0.82
	40%	0.6±0.1	2.1±0.1	71.4	0.1±0.0	0.7±0.1	85.7	1.16
	60%	0.6±0.0	2.5±0.3	76	0.1±0.0	0.9±0.1	88.8	1.31
Photoperiod	8 hours	0.6±0.0	1.7±0.1	64.7	0.1± 0.0	0.6±0.1	83.3	0.82
	12 hours	0.6±0.0	2.5±0.2	76	0.1±0.0	0.9±0.2	88.8	1.31
	24 hours	0.6±0.0	2.6±0.3	76.9	0.2±0.1	0.8±0.1	75	1.06
		<i>P. sphenops</i>						
Feeding frequency	1	0.6±0.0	2.3 ±0.2	73.9	0.2 ±0.0	0.7 ±0.1	71.4	0.55
	2	0.6±0.0	3.0 ±0.1	80	0.2 ±0.0	1.1 ±0.1	81.8	0.75
	3	0.6±0.0	3.2 ±0.1	81.25	0.2 ±0.0	1.2 ±0.1	83.3	0.79
Feed protein content	20%	0.6±0.0	1.7±0.1	64.7	0.1±0.0	0.5±0.1	80	0.96
	40%	0.6±0.0	2.5±0.1	76	0.1±0.0	0.8±0.1	87.5	0.96
	60%	0.6±0.1	2.7±0.1	77.7	0.1±0.0	1.0±0.1	90	1.24
Photoperiod	8 hours	0.6±0.1	1.5±0.1	60	0.1±0.0	0.4±0.1	75	0.82
	12 hours	0.6±0.1	2.7±0.2	77.7	0.1±0.0	1.0±0.1	90	1.16
	24 hours	0.6±0.0	2.1±0.1	71.4	0.2±0.1	0.7±0.1	71.4	1.37

experimental fish after one week of stocking in each salinity treatment was recorded.

Effect of photoperiod: The effect of photoperiod on growth and survival of *P. sphenops* and *P. reticulata* was estimated subjecting experimental fish into three different photoperiods, 8, 12 and 24 hours per day (Boeuf and Le Bail, 1998; Biswas et al., 2006). This experiment was carried out for 24 weeks and at the end of the experiment, the growth and survival of fish were estimated with respect to each treatment.

Effect of sex ratio on fecundity: Three different female: male sex ratios; 2:1, 3:1 and 3:2 of *P. sphenops* and *P. reticulata* species were maintained for a period of 40 weeks. Aquarium bred brooders of similar age (6 months old) and size (1.2±0.2 g) were reared in 55×30×30 cm glass tanks under laboratory conditions. The number of offspring produced under each sex ratio was counted and recorded.

Data analysis: In growth experiments, percentage body weight gain (BWG%), percentage length gain (LG%) and specific growth rate (SGR) were calculated following the formula described by Anka et al. (2016).

Percentage Body weight gain (BWG%)

$$= \frac{W_t - W_0}{W_t} \times 100\%$$

Percentage length gain (LG%)

$$= \frac{L_t - L_0}{L_t} \times 100\%$$

Specific growth rate (SGR) (%/day)

$$= (\ln W_t - \ln W_0) \times 100 t^{-1}$$

Where, W_t and W_0 were mean final and initial fish weight (g), L_t and L_0 were mean final and initial length (cm), respectively and t was the experimental duration in days. The survival was calculated according to the following equation,

$$\text{Survival (\%)} = N_t \times 100 N_0^{-1}$$

Where, N_t and N_0 were final and initial numbers of fry in each treatment, respectively. Variations in the average growth parameters of fish subjected to different treatments of feeding frequency, feed protein level, larval feed, salinity and photoperiod were compared using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The effect of sex ratio on fecundity of *P. sphenops* and *P. reticulata* was also assessed using ANOVA. Differences were considered to be

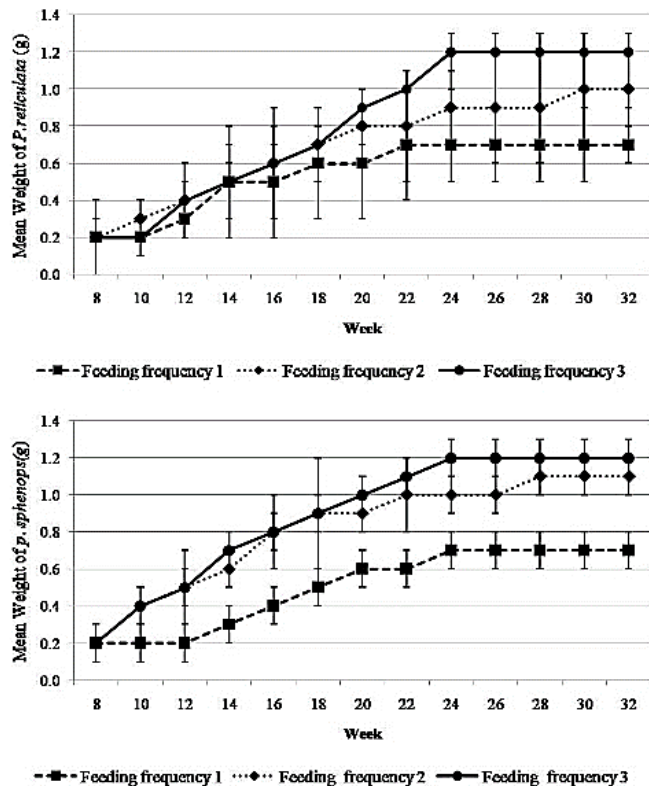


Figure 2. Variations in mean weight (\pm SD g) of *Poecilia reticulata* (above) and *P. sphenops* (below) reared under three different feeding frequencies.

significant when $P < 0.05$. All the statistical tests were performed in Minitab 14 for Windows statistical package.

Results

Effect of feeding frequency: Variations in weight (mean \pm SD g) of *P. sphenops* and *P. reticulata* and reared in three different feeding frequency treatments were compared (Fig. 2). In both these species, experimental fish fed thrice a day reported the highest average weight as well as the highest specific growth rate (SGR) while the fish fed with one time per day reported the lowest values (Fig. 2, Table 2). However, there was no significant impact of feeding frequency on weight gain of these species ($P > 0.05$, ANOVA, $df = 2$). At the end of this experiment, fish showed 100% survival in all the experimental tanks.

Effect of feed protein content: The feed protein level has a significant impact on growth of *P. reticulata* and *P. sphenops* (Fig. 3). Fish fed with 20% protein feed showed significantly lower weight gain than the 40%

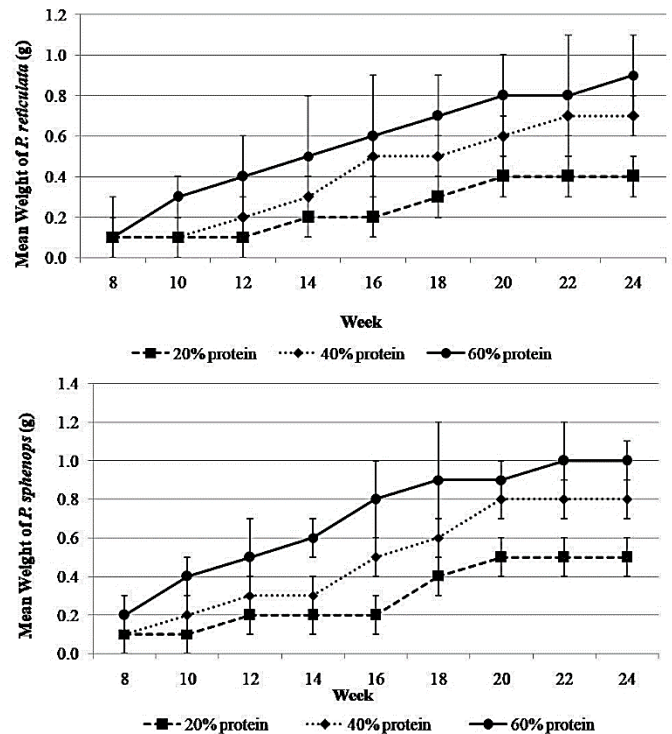


Figure 3. Variations in mean weight (\pm SD g) of *Poecilia reticulata* (above) and *P. sphenops* (below) reared under three different feed protein levels.

and 60% protein feeds ($P < 0.05$, ANOVA, $df = 2$). The lowest specific growth rate was also evident in the fish fed with 20% protein feed (Table 2). However, experimental fish showed 100% survival at the end of this experiment.

Effect of larval feeds: Fry of *P. reticulata* and *P. sphenops* fed with newly hatched *Artemia* showed a higher mean weight than the other two larval feeds (Fig. 4). However, there was no significant impact of feed types on growth and survival of early life stages of *P. reticulata* and *P. sphenops* ($P > 0.05$, ANOVA, $df = 2$).

Effect of salinity: *P. reticulata* fry showed 100% survival in 5 and 10 ppt saline water but they showed only 40% survival in 15 and 20 ppt saline water. However, adult *P. reticulata* reported 100% survival up to 20 ppt salinity and 60% survival in 25 ppt salinity. They survived only 2 days in 30 ppt salinity. Both fry and adults of *P. sphenops* could tolerate salinity up to 10 ppt. Although 100% survival of adult *P. sphenops* was recorded in 10 ppt saline water, only 80% of fry were survived. Both adults and fry

Table 3. Number of offspring produced by *Poecilia reticulata* and *P. sphenops* under different sex ratios during the experimental period.

Sex Ratio (Female : Male)	Number of Offspring produced	
	<i>P. reticulata</i>	<i>P. sphenops</i>
2:1	130	97
3:1	178	162
3:2	526	352

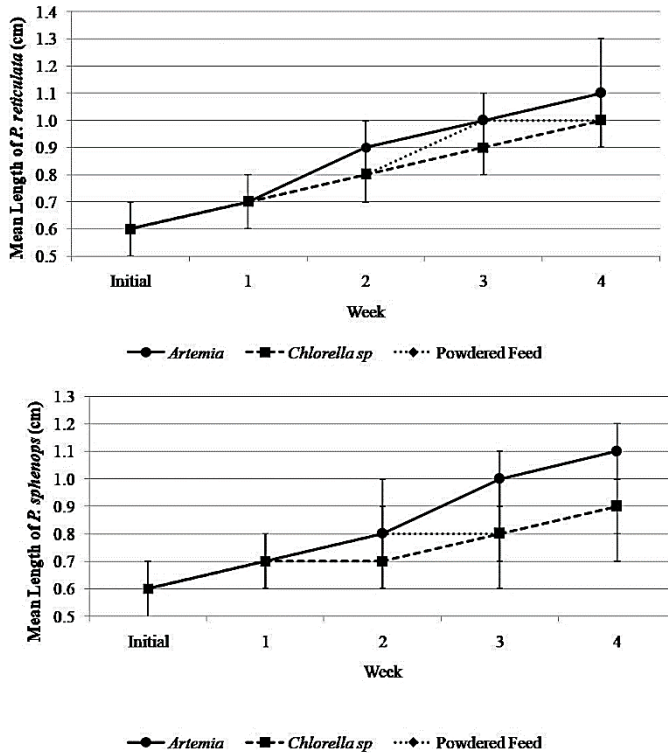


Figure 4. Variations in mean length (\pm SD g) of *Poecilia reticulata* (above) and *P. sphenops* (below) fry reared under three different larval feeds.

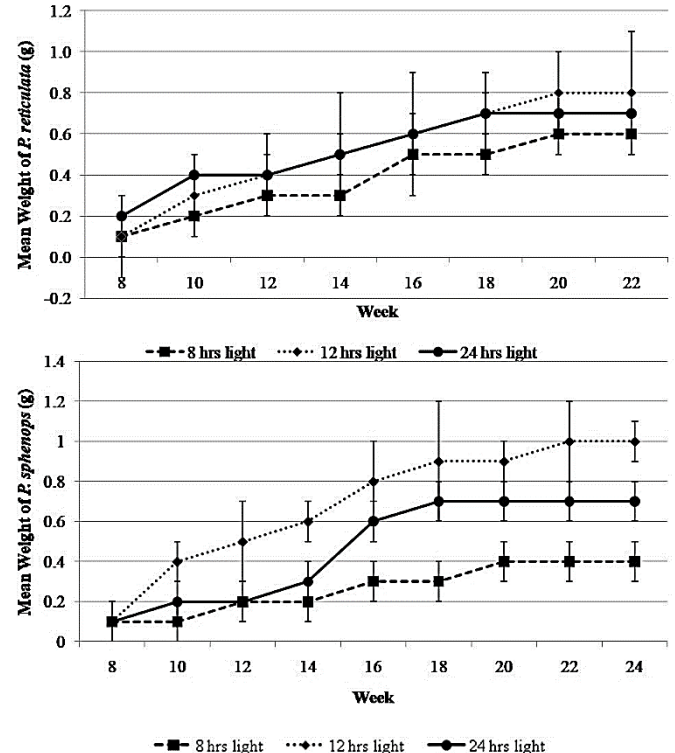


Figure 5. Variations in mean weight (\pm SD g) of *Poecilia reticulata* (above) and *P. sphenops* (below) reared in different photoperiods.

($P < 0.05$, ANOVA, $df = 2$). (Table 3).

Discussions

Although Sri Lanka is considered as one of the pioneers in ornamental fish industry in the world, very few scientific studies have been conducted to identify the appropriate culture conditions for ornamental fish species. Therefore, this study was carried out to identify the most suitable conditions for the growth and survival of *P. reticulata* and *P. sphenops* under aquarium conditions.

In feeding frequency experiments, the observed higher specific growth rate of fish fed 2 and 3 times per day compared to the fish fed once a day, could be probably due to fish reaching to the level of satisfaction or to the level of satiation when they were

survived less than 24 hours in 15 ppt saline water.

Effect of photoperiod: A significant impact on photoperiod on growth of *P. sphenops* and *P. reticulata* was reported (Fig. 5). Experimental fish exposed for 8 hours photoperiod showed significantly lower weight gain than the fish exposed for 12 and 24 hours photoperiods ($P < 0.05$, ANOVA, $df = 2$). Both species reared under 12 hours photoperiod showed the highest SGR (Table 2). A survival rate of 100% was recorded in all photoperiod experiments.

Effect of sex ratio on fecundity: A total of 834 *P. reticulata* and 611 *P. sphenops* offspring were produced during the study period. In both these species, a significantly higher number of offspring were reported when (female:male) sex ratio was 3:2

fed 2 or 3 times a day (Grayton and Beamish, 1977). Though, feeding fish thrice a day ensures the highest SGR of both species, a comparatively high accumulation of waste materials, including both excretory products of fish and uneaten feed particles and discolored turbid water was observed in all experimental tanks. Therefore, more time and labor was required and the wastage of food was high in feeding frequency 3 treatment. As the feeding of fish considered to occupy 60-70% of total cost of production in ornamental aquaculture (Rathnayake et al., 2016), feeding fish 2 times a day can be considered as the most economically feasible feeding frequency which ensures the optimum growth of *P. reticulata* and *P. sphenops* under aquarium conditions. As the survival rate of fish in all treatments was 100%, it confirms that there is no any effect of feeding frequency on the survival of *P. reticulata* and *P. sphenops*.

Feed protein plays a major role in the growth of fish and it has been reported that fish requires two to four times more dietary protein compared to warm-blooded animals, as they need relatively higher level of essential amino acids (Sumithra et al., 2015). On dry weight basis, protein contributes about 65-75% of the total weight of fish tissue (Shim and Chua, 1986). It is reported that decrease of body weight can occur when fish are fed with low dietary protein levels (Dabrowski, 1977). In feed protein experiment, 20% feed protein level showed a significantly lower growth in *P. reticulata* and *P. sphenops* compared to 40 and 60% feed protein levels. The reduced growth in fish fed with 20% or less protein levels also have observed by Shim and Chua (1986). The dietary protein is considered to be just enough for growth and repair as the protein sources are more expensive than carbohydrates and fat sources (Sumithra et al., 2015). As the fish fed with feed containing 40 and 60% dietary protein levels showed no significant difference in growth of *P. reticulata* and *P. sphenops*, 40% dietary protein level can be proposed as the ideal protein level in commercial fish feed due to cost effectiveness.

Although there was no any significant impact of

feed types on growth and survival of *P. reticulata* and *P. sphenops* fry, the results revealed that fry fed with newly hatched *Artemia* attained the highest mean weight followed by the fry fed with commercial larval feed and *Chlorella* sp.. It was reported that newly hatched *Artemia* contain high level of nutrients; especially they are rich with proteins (Leger et al., 1991). Further, nutrient loss from live feeds like *Artemia* is much less or negligible when compared with formulated artificial diets (Goldblatt et al., 1980). Furthermore, *Artemia* does not deteriorate the water quality by accumulating in the bottom when compared to commercial fish feed (Harpaz et al., 2005). Because of these favorable factors, when *Artemia* are given to fry they can easily detect their food, get comparatively long feeding time and receive high protein diet, which finally enhance their growth. The observed reduced mean weight in fry fed with *Chlorella* sp. can be due to the lack of animal protein in this feed (Dussault and Kramer, 1980).

Poeciliids are generally active fish during daylight (Meffe and Snelson, 1989). Therefore, they have a clear connection with the photoperiod. The observed significant reduction in growth of *P. reticulata* and *P. sphenops* subjected to 8 hours photoperiod per day could be mainly due to their reduced feed intake. As a diurnally active fish species, Poeciliids actively feed during daylight. When photoperiod is reduced, the amount of feed intake by these species is also reduced causing their growth retardation (Biswas et al., 2006). High accumulation of excess feed in the experimental tanks which were subjected to 8 hours photoperiod than 12 and 24 hours photoperiods may be a result of reduced feeding rate during dark. But the survival of these species was not affected by the photoperiods.

Some fish are highly euryhaline but they do not use their ability to tolerate seawater in the natural environment. Similarly, some species belonging to family Poeciliidae are euryhaline though they live mostly in freshwater environments (Rosen and Bailey, 1963). The experiment recorded survival of *P. reticulata* in 25 ppt saline waters which also has been observed previously by Shikano and Fujio (1998). According to Pisam et al. (1987), acclimation

to the seawater can be induced by developing osmoregulatory functions such as branchial chloride cells in euryhaline fish including Poeciliids. But due to unknown reasons *P. sphenops* showed comparatively lesser salinity tolerance compared to *P. reticulata* in this experiment.

In both experiments a significant high fecundity was observed when female to male sex ratio was maintained as 3:2 than 2:1 and 3:1. The exact reason for this result is unknown but it might be the high number of males in the replicates which results a higher sperm production (Evens and Magurran, 1999).

Conclusion

The study recommends several culture conditions for *P. reticulata* and *P. sphenops* namely, maintaining a feeding frequency of twice per day, dietary protein level of 40%, 12 hours photoperiod and female: male sex ratio of 3:2. The impact of the type of fry feed on the growth and survival was not significant. *Poecilia sphenops* and *P. reticulata* could tolerate salinity up to 10 and 25 ppt, respectively.

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