Effects of pH, feeding regime and thyroxine on growth and survival of *Parosphromenus tweediei* (Kottelat and Ng, 2005) fry

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Abstract

Parosphromenus tweediei is a rare native fish of Malaysia. It is found only in peat swamp areas. Being traded at high market price of MYR40 per fish shows its potential as ornamental fish. Currently, this fish is threatened to extinction due to anthropogenic activities. Particularly when peat swamps are converted into paddy, pineapple or oil palm plantations. To date, there are very few studies on *P. tweediei*. Culture of this species is non-existent. Therefore, the objectives of this study were to look into several factors affecting the growth and survival of this species in control condition. Aspects studied were on the effect of pH, feeding regime and thyroxine (T_4) on growth and survival of *P. tweediei* fry. Experiments were carried out at Hatchery Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Parameters for water quality such as dissolved oxygen, temperature, ammonia, nitrite and nitrate were monitored once a week throughout the experimental period. All experiments were conducted using polystyrene box with size of 34x21x19cm. Experiment on pH consisted of pH 4.5, 5.0, 5.5 and 6.0, which were prepared in triplicates with stocking density of 12 fry per replicate. As for experiment on feeding regime, three regimes used were AM: *Artemia* nauplii (6-20DAH) - *Moina* sp. (21-63DAH), AMG1: *Artemia* nauplii (6-20DAH) - *Moina* sp. (21-43DAH) - Grindal worm (44-63DAH) and AMG2: *Artemia* nauplii (6-20DAH) - *Moina* sp. (27-48DAH) - Grindal worm (49-63DAH). All treatments were triplicated with 10 fry per tank. As for T_4 experiment, 3 dosages tested were 0, 0.5 and 1 ppm, conducted in duplicates with 10 fry per tank. Immersions of T_4 on 28 DAH fry were carried out only once at the beginning of the experiment. Results showed that fry of *P. tweediei* can be successfully cultured from pH 4.5 to 6.0. It was observed that total length (TL) and survival increase as pH level decreases from 6.0 to 4.5. As for feeding regime, statistical analysis showed that there were significant differences (p<0.05) among treatments, with AMG2 produced the highest TL (18.91 mm) as compared to the other two treatments. As for T_4 experiment, T_4 at 0.5ppm produced the highest (p<0.05) TL as compared to 0 and 1.0ppm. Nevertheless, the percentage of survival and water quality were not significantly different (p>0.05) among the treatments in both feeding regime and thyroxine experiments. As a conclusion, *P. tweediei* fry grow and survive best in acidic water condition. In additions, a single application of T_4 at 0.5 ppm is sufficient to induce the growth of *P. tweediei* at early stage.

Keywords: pH, Feeding regime, Thyroxine, *Parosphromenus tweediei*, Total length, Survival

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Introduction

Growth and development of fish are influence by environmental factors and food availability. Maintenance of water quality with suitable water requirements will enhance the survival and growth of the fish. Fish continuously grow as they live and relied on external environment (Brett, 1979; Boeuf *et al*., 1999). The combination of several factors like nutrition, substrate, water quality and stress affect the survival of the fish (Herath and Atapaththu, 2012). These ecological factors can be divided into physical and chemical factor. Physical factor includes temperature, salinity, and photoperiod. While the chemical factor encompassed ammonia and pH.

Chemical factor like pH is very crucial in fish culture. It is an indicator of acidic or alkaline condition. Water considered acidic when pH is below 7 and considered alkaline when above 7. In aquaculture, pH between 6.5 and 8.5 is considered as the most suitable range and recommended as a guideline for proper practice (Summerfelt, 1998). However, this may not be applicable for certain species of fish. Suitable pH conditions prevent metabolic stress and help fish efficiently utilized nutrients from the feed and at the same time optimize digestion, thereby maximizing the growth of the fish (Bolner and Baldisserto, 2007; Rebouças *et al*., 2015). Based on their natural habitat, *P. tweediei* thrive well in an acidic condition. In order to gauge the water parameters tolerance of the species, a study was conducted to compare the growth and survival of *P. tweediei* fry cultured at different pH.

The availability and quality of feeds are also important factors contributing to decent growth performance and successful breeding of ornamental fish (Degani and Yehuda, 1996). During nursery stage, the lack of suitable food causes low survival of fry (Chaudhuri, 1979). Often fish breeders use live food in the culture of ornamental fish. It is use to induce ornamental fishes to breed (Shelar *et al*., 2014). Studies also revealed that fishes have better growth and survival when fed with live food (Srikrishan *et al*., 2017). Live food provides the required nutrients as they are ready for consumption and efficiently digested to support growth of fish larvae (Kumar *et al*., 2008). Commonly used live food in ornamental fish culture is *Artemia*, *Moina*, copepods, *Brachionus* and *Chrinomus* (Herath and Atapaththu, 2012). In recent years, *Artemia* has extensively used as live food since it ensures better growth and survival of fish fry. However, *Artemia* is quite costly (Lim *et al*., 2003). So, identification of low cost live food is needed as to reduce the production cost as well as to ensure sustainability of the fish production. Therefore, the second experiment evaluates the effect of different feeding regimes on growth and survival of *P. tweediei*.

Growth hormone is important during early life stage of fish. It plays an important role since high level of thyroid hormone is found in eggs, and it acts as promoting agent for larva-juvenile transition (Yamano, 2005). Thyroid

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hormone contained tri-iodothyronine (T_3) and prohormone thyroxine (T_4). T_3 and T_4 are widely used in animal husbandry and play an important role in the application for vertebrate, since it can influence metabolism (Gorbman, 1969). Several studies showed the effect of T_3 and T_4 application onto eggs and fish larvae. It enhances growth and survival of tilapia, conger eel, sea bream, striped bass, rabbit fish, coho salmon, sockeye salmon and pink salmon (Power e*t al.*, 2001). As growth factor in fish is very important, this led to the determination of T_4 effect on the growth and survival of *P. tweediei* fry. This hormone can be applied on fish through immersion, injection or incorporated into feed (Higgs and Eales, 1973; Eales, 1974; Hurlburt, 1977).

Material and methods

Location of study

Study was conducted at the Hatchery Unit, Institute of Biosciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Adults *Parosphromenus tweediei* (Fig. 1) were caught from the wild along roadside canal at the vicinity of Sri Bunian, Pontian, Johor. Three water parameters measured at the natural habitat were pH, temperature and dissolved oxygen.

Breeding trial

Broodstocks were conditions for 3 weeks prior to breeding. Fishes with total length of 2.3-2.7cm, with a ratio of 1 male: 1 female was used. Breeding tank with size 26x15x15 cm was prepared with the addition of Java Fern, *Microsorum pteropus* attached to drift wood and 5cm diameter PVC pipe (7-12cm length). The side of the tank was covered with black plastic to reduce the penetration of light. Peat granules (JBL Tormec Activ) were soaked in the water to get the desired pH for breeding. All tanks were prepared one week before fish was placed inside. The tanks were monitored in the morning and evening in order to observe the presence of eggs. Male usually makes bubble nest in the inner surface of the PVC pipe. Upon successful spawning, eggs were kept in the breeding tank until hatched and at 3 days after hatch (DAH), all the postlarvae attached to the inner side of the PVC pipe, were removed into the larval rearing tanks. At this time, postlarvae were fed with green water taken from tilapia pond. This green water consisted of *Monoraphidium* sp., *Closterium* sp., *Klebsormidium* sp., *Chlorella* sp. and *Scenedesmus* sp.

Figure 1: Adult *Parosphromenus tweediei*

pH Tolerance

Culture water was prepared from dechlorinated tap water into pH 4.5, 5.0, 5.5, 6.0, and prepared in triplicates with

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stocking of 12 fry per replicate. All the pH values were achieved using peat granule, JBL Tormec Activ. Seven day after hatch (DAH) fry were placed in polystyrene box size 34x21x19cm and observed weekly until 63 DAH. Water change of 10% was carried out weekly.

Feeding regime

Three feeding regimes used in this study were AM, AMG1 and AMG2. The first feeding regime AM consisted of *Artemia* nauplii given from 6 DAH, then followed by *Moina* sp. at 21 DAH onwards. The second feeding regime, AMG1 consisted of *Artemia* nauplii from 6 DAH, then *Moina* sp. from 21 DAH and grindal worm, *Enchytraeus buchholzi* from 44 DAH onwards. The third feeding regime, AMG2 consisted of *Artemia* nauplii given from 6 DAH, and then followed by *Moina* sp. from 28 DAH and then grindal worm, *Enchytraeus buchholzi* from 49 DAH onwards. Each treatment was triplicated with stocking of 10 fry per replicate. Feeding regime experiment was conducted after the pH experiment. Therefore, the pH of culture water used in this experiment was based on the best result from the pH experiment. Water changes of 10% were carried out weekly.

Thyroxine preparation

As for thyroxine (T_4) experiment, 3 dosages used were 0, 0.5 and 1 ppm. Hormone immersions were carried out for 30 minutes at the initiation of experiment on fry of 28 DAH. Fry were stocked into polystyrene box measuring 34x21x19cm. L-thyroxine or T_4 powder (Sigma Chemical, USA) was dissolved in distilled water containing 0.06% NaCl per liter and pH was adjusted between pH 8.0 to 8.5 with 1 *N* alkali and 0.1 *N* HCl (Mareedu and Gudamani, 2013). Each treatment tank was duplicated with stocking of 10 fry per replicate. Similar to feeding regime experiment, the pH of water used in this experiment was based on the best pH from the first experiment. Water changes of 10% were carried out weekly.

Total length and Survival

Sampling for total length (TL) of fry was carried out, once a week to obtain the average length. Total length was measured from the tip of the mouth to the caudal fin of the fish (Fishbase, 2004). The lengths were measured using digital calliper (Mitutoyo, Thailand). Mortality of fry was observed and noted throughout the experimental period.

Water quality measurements

Water pH was measured using pH pen (Hanna, Romania). While temperature (^oC) and dissolved oxygen, DO (mg/L) were measured using YSI Multi-Parameter. While, API freshwater master test kit was used to determine the ammonia, nitrite and nitrate levels in the culture tank. All the parameters were measured once a week, prior to 10 % water change.

Feeding

All the broodstocks and the fry were fed to observed satiation twice daily at 0800 and 1700. Grindal worm, *Enchytraeus*

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buchholzi, *Artemia* nauplii (INVE Aquaculture Inc., Belgium) and sometimes *Daphnia magna* neonates were used as food for the broodstock. While, green water and microworm, and *Panagrellus redivivus* were fed to the fry from the age of 3 DAH. Except for feeding regime experiment, fry and juveniles were given mixture of *Artemia* nauplii and grindal worm, *Enchytraeus buchholzi*. Uneaten food and faecal matters were removed by siphoning. All the live foods were produced in the laboratory.

Statistical analysis

Data were presented in mean ± standard deviation (SD). All statistical analyses were performed using SPSS 22.0. Data for total length were analysed statistical by using one way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests. All data in percentage were transformed using arcsine before being used for ANOVA. All data were keyed into Microsoft Excel and then transferred to the SPSS software for statistical analysis.

Results

pH tolerance

Parosphromenus tweediei fry at 7 DAH with initial TL of 4.71 mm were used for pH experiment until 63 DAH. Water quality sampled from the natural habitat showed that this species can thrive well in pH 4.3 to 5.9 (Table 1). Thus the pH tested in this experiment was 4.5, 5.0, 5.5, and 6.0.

Table 1: Water quality of the natural habitat of *Parosphromenus tweediei* at Sri Bunian, Pontian, Johor.

Water parameters	Range
pH	4.3 - 5.9
Temperature (˚C)	24.4 - 26.7
Dissolved oxygen, DO (mg/L)	6.52 - 8.04

Significant differences (p<0.05) were observed in total length (TL) for *P. tweediei* fry cultured at pH 4.5, 5.0, 5.5 and 6.0 (Table 2). At early stage of the experiment, at 14 DAH, *P. tweediei* fry cultured in pH 4.5 and 5.0 showed significantly (p<0.05) better TL as compared to pH 5.5 and 6.0. *Parosphromenus tweediei* fry at 21 DAH, in pH 4.5 showed highest (p<0.05) TL followed by fry in pH 5.0 and then fry in pH 5.5 and 6.0. However, fry at 28 DAH, showed significantly highest (p<0.05) TL, followed by fry at pH 4.5, then pH 5.0, then 5.5 and finally at pH 6.0. Similar results were observed for fry at 35, 42, 49, 56 and 63 DAH. At 63 DAH, fry achieved final TL of 18.93, 17.85, 15.22 and 14.70 mm for pH 4.5, 5.0, 5.5 and 6.0 respectively.

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Table 2: Mean of total length (TL) and survival of *Parosphromenus tweediei* fry at different pH during the 56 days experimental period.

Day after hatch (DAH)	Parameter	pH			
		4.5	**5.0**	**5.5**	**6.0**
7 (Initial)	TL (mm)	4.71 ± 0.08^{a}	4.71 ± 0.09^{a}	4.71 ± 0.05^{a}	4.71 ± 0.04^{a}
14	TL (mm)	5.52 ± 0.06^{b}	5.46 ± 0.03^{b}	5.24 ± 0.03^{a}	5.23 ± 0.03^{a}
	Survival (%)	100 ± 0.00^{a}	100 ± 0.00^{a}	94.44 ± 0.23^{a}	91.67 ± 0.28^{a}
21	TL (mm)	6.76 ± 0.04^{c}	6.50 ± 0.04^{b}	6.22 ± 0.03^{a}	6.16 ± 0.02^{a}
	Survival (%)	100 ± 0.00^{b}	100 ± 0.00^{b}	88.89 ± 0.32^{ab}	80.56 ± 0.40^{a}
28	TL (mm)	8.02 ± 0.01^{d}	7.73 ± 0.03^{c}	7.27 ± 0.06^{b}	7.11 ± 0.05^{a}
	Survival (%)	100 ± 0.00^{b}	100 ± 0.00^{b}	83.33 ± 0.38^{ab}	77.78 ± 0.42^{a}
35	TL (mm)	9.55 ± 0.05^{d}	9.15 ± 0.06^{c}	8.33 ± 0.09^{b}	8.10 ± 0.06^{a}
	Survival (%)	100 ± 0.00^{b}	100 ± 0.00^{b}	77.78 ± 0.42^{a}	75.00 ± 0.44^{a}
42	TL (mm)	11.49 ± 0.01^{d}	10.86 ± 0.03^{c}	9.52 ± 0.03^{b}	9.32 ± 0.04^{a}
	Survival (%)	100 ± 0.00^{b}	100 ± 0.00^{b}	77.78 ± 0.42^{a}	77.78 ± 0.42^{a}
49	TL (mm)	13.64 ± 0.06^{d}	12.95 ± 0.06^{c}	11.10 ± 0.04^{b}	10.93 ± 0.04^{a}
	Survival (%)	100 ± 0.00^{b}	100 ± 0.00^{b}	77.78 ± 0.42^{a}	77.78 ± 0.42^{a}
56	TL (mm)	16.12 ± 0.02^{d}	15.25 ± 0.03^{c}	13.10 ± 0.01^{b}	12.72 ± 0.01^{a}
	Survival (%)	100 ± 0.00^{b}	100 ± 0.00^{b}	77.78 ± 0.42^{a}	77.78 ± 0.42^{a}
63	TL (mm)	18.93 ± 0.01^{d}	17.85 ± 0.01^{c}	15.22 ± 0.02^{b}	14.70 ± 0.02^{a}
	Survival (%)	100 ± 0.00^{b}	100 ± 0.00^{b}	77.78 ± 0.42^{a}	77.78 ± 0.42^{a}

Mean values with the same superscripts within the same row are not significantly different (p>0.05); ± SD

There were no significant differences (p>0.05) in survival of 7 – 14 DAH fry. At 21 DAH, fry survival was highest in pH 4.5 followed by pH 5.0, and the lowest (p<0.05) in pH 5.5 and 6.0.At 28 DAH, survival of fry was highest (p<0.05) in pH 4.5 and 5.0 as compared to pH 6.0. However, these percentages of survival were not significantly different (p>0.05) when compared to pH 4.5, 5.0 and 5.5. At 35 DAH, fry survival was higher (p<0.05) in pH 4.5 and 5.0 as compared to pH 5.5 and 6.0. Similarly for fry survival from 42 to 63 DAH. Overall, the survival of *P. tweediei* fry ranged from 77.8 - 100% between treatments by the end of the experimental period with higher (p<0.05) survival of 63 DAH fry in pH 4.5 and 5.0 as compared to fry in pH 5.5 and 6.0. These findings suggested that *P. tweediei* fry is most suitable in acidic environment evident with the better growth and survival at lower pH.

Water quality throughout the culture period is as shown in Table 3. Mean values for DO and temperature were not significantly different (p>0.05) between pH 4.5, 5.0, 5.5 and 6.0. However ammonia and nitrite were significantly higher (p<0.05) in pH 4.5 and 5.0 as compared to pH 5.5 and 6.0. While nitrate was highest (p<0.05) in pH 4.5, followed by pH 5.0 and 5.5, then pH 6.0. Ranges of DO and temperature monitored during this study were 3.05 – 4.65 mg/L and 25 – 27°C respectively.

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While ranges for ammonia, nitrite and nitrate were 0.25 -1.00 ppm, 0 1 ppm and 0 – 5 ppm, respectively.

Table 3: Water quality parameters in culture tank at different pH throughout the 56 days experimental period

Water Parameter	pH			
	4.5	5.0	5.5	6.0
Dissolved oxygen (mg/L)	3.88 ± 0.10[a]	3.69 ± 0.02[a]	3.85 ± 0.03[a]	3.84 ± 0.11[a]
Range	3.50 - 4.50	3.05 - 3.90	3.82 - 4.15	3.75 - 4.65
Temperature (°C)	25.45 ± 0.11[a]	25.31 ± 0.43[a]	25.58 ± 0.56[a]	25.71 ± 0.31[a]
Range	25.5 - 27.0	25.5 - 27.0	25.0 - 27.0	25.0 - 27.0
Ammonia (ppm)	0.65 ± 0.07[b]	0.59 ± 0.08[b]	0.42 ± 0.03[a]	0.27 ±0.03[a]
Range	0.50 – 1.00	0.25 - 1.00	0.25 - 0.50	0.25 - 0.50
Nitrite (ppm)	0.35 ± 0.03[b]	0.38 ± 0.00[b]	0.17 ± 0.02[a]	0.19 ± 0.03[a]
Range	0.00 - 1.00	0.00 - 0.50	0.00 - 0.25	0.00 - 0.50
Nitrate (ppm)	3.54 ± 0.36[c]	2.50 ± 0.00[b]	2.50 ± 0.00[b]	1.67 ± 0.36[a]
Range	0.00 - 5.00	0.00 - 5.00	0.00 - 5.00	0.00 - 5.00

Mean values with the same superscripts within the same row are not significantly different (p>0.05); ± SD.

Feeding regime

Results from the feeding regime experiment showed that this factor affected the growth of the fry. Feeding regimes include AM: *Artemia* nauplii (6-20 DAH) and *Moina* sp. (21- 63DAH), AMG1: *Artemia* nauplii (6-21DAH), *Moina* sp. (21-43DAH) and grindal worm (44-63DAH), and AMG2: *Artemia* nauplii (6-20DAH), *Moina* sp. (27-48DAH) and grindal worm (49-63DAH). Based on the best pH from the previous experiment, water pH for all tanks in this experiment was maintained at pH 4.5 throughout the 56 days experimental period. The initial TL of fry at 7 DAH was 4.92 mm (Table 4). At 14 and 21 DAH there was no significant different (p>0.05) in TL of fry between treatments. But at 28 DAH, AMG2 showed significantly higher (p<0.05) TL of fry as compared to AM and AMG1. Similar results were observed in fry of 35, 42 and 49 DAH. While at 56 and 63 DAH, TL of fry fed with AMG2 were highest (p<0.05) at 18.91 mm, followed by AMG1 at 18.24 mm, and lowest (p<0.05) 17.97 mm when using AM. Survival of *P. tweediei* fry ranged from 93.33 to 96.67% were not significantly different (p>0.05) between all feeding regimes throughout the 56 days experimental period. Results showed that all feeding regimes are able to maintain good survival; however feeding regime of AMG2 is more than sufficient to support good growth of *P. tweediei* fry.

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Table 4: Mean of total length (TL) and survival of *Parosphromenus tweediei* fry with different feeding regime for 56 days experimental period.

Day after hatch (DAH)	Parameter	Feeding Regime		
		AM	AMG1	AMG2
7 (Initial)	TL (mm)	4.92 ± 0.03[a]	4.92 ± 0.03[a]	4.92 ± 0.05[a]
14	TL (mm)	5.70 ± 0.03[a]	5.71 ± 0.07[a]	5.72 ± 0.08[a]
	Survival (%)	100.00 ± 0.00[a]	96.67 ± 0.18[a]	96.67 ± 0.18[a]
21	TL (mm)	6.90 ± 0.02[a]	6.91 ± 0.03[a]	6.91 ± 0.02[a]
	Survival (%)	100.00 ± 0.00[a]	96.67 ± 0.18[a]	96.67 ± 0.18[a]
28	TL (mm)	8.02± 0.03[a]	8.01 ± 0.01[a]	8.22 ± 0.02[b]
	Survival (%)	96.67 ± 0.18[a]	93.33 ± 0.25[a]	96.67 ± 0.18[a]
35	TL (mm)	9.33 ± 0.06[a]	9.30 ± 0.04[a]	9.61 ± 0.02[b]
	Survival (%)	96.67 ± 0.18[a]	93.33 ± 0.25[a]	96.67 ± 0.18[a]
42	TL (mm)	11.15 ± 0.02[a]	11.16 ± 0.02[a]	11.60 ± 0.02[b]
	Survival (%)	96.67 ± 0.18[a]	93.33 ± 0.25[a]	96.67 ± 0.18[a]
49	TL (mm)	13.23 ± 0.02[a]	13.25 ± 0.02[a]	13.82 ± 0.02[b]
	Survival (%)	96.67 ± 0.18[a]	93.33 ± 0.25[a]	96.67 ± 0.18[a]
56	TL (mm)	15.32 ± 0.04[a]	15.64 ± 0.03[b]	16.26 ± 0.05[c]
	Survival (%)	96.67 ± 0.18[a]	93.33 ± 0.25[a]	96.67 ± 0.18[a]
63	TL (mm)	17.97 ± 0.02[a]	18.24 ± 0.01[b]	18.91 ± 0.02[c]
	Survival (%)	96.67 ± 0.18[a]	93.33 ± 0.25[a]	96.67 ± 0.18[a]

Mean values with the same superscripts within the same row are not significantly different (p>0.05); ±SD.
AM: *Artemia* nauplii (DAH6-20) - *Moina* sp. (DAH21-63)
AMG1: *Artemia* nauplii (DAH6-20) - *Moina* sp. (DAH21-43) - Grindal worm (DAH44-63)
AMG2: *Artemia* nauplii (DAH6-20) - *Moina* sp. (DAH27-48) - Grindal worm (DAH49-63)

Table 5: Water quality parameters in culture tank at different feeding regimes throughout the 56 days experimental period

Water Parameter	Feeding regime		
	AM	AMG1	AMG2
Dissolved oxygen (mg/L)	3.91 ± 0.00[a]	3.91 ± 0.02[a]	3.91 ± 0.01[a]
Range	3.75 - 4.65	3.75 - 4.65	3.75 - 4.65
Temperature (°C)	25.36 ± 0.04[a]	25.36 ± 0.15[a]	25.40 ± 0.12[a]
Range	25.0 - 26.0	25.0 - 26.0	25.0 - 26.0
Ammonia (ppm)	0.71 ± 0.01[a]	0.71 ± 0.01[a]	0.71 ± 0.01[a]
Range	0.25 - 1.00	0.25 - 1.00	0.25 - 1.00
Nitrite (ppm)	0.45 ± 0.05[a]	0.44 ± 0.04[a]	0.40 ± 0.01[a]
Range	0.00 - 0.50	0.00 - 0.50	0.00 - 0.50
Nitrate (ppm)	4.76 ± 0.04[a]	4.76 ± 0.05[a]	4.72 ± 0.01[a]
Range	0.00 – 5.00	0.00 – 5.00	0.00 – 5.00

Mean values with the same superscripts within the same row are not significantly different (p>0.05); ±SD.
Water pH in all tanks were maintained at 4.5 throughout the experimental period
AM: *Artemia* nauplii (DAH6-20) - *Moina* sp. (DAH21-63)
AMG1: *Artemia* nauplii (DAH6-20) - *Moina* sp. (DAH21-43) - Grindal worm (DAH44-63)
AMG2: *Artemia* nauplii (DAH6-26) - *Moina* sp. (DAH27-48) - Grindal worm (DAH49-63)

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Mean values for DO, temperature, ammonia, nitrite, nitrate were not significantly different (p>0.05) between all feeding regimes (Table 5).

Ranges of DO, water temperature, ammonia, nitrite and nitrate during the experimental period were 3.75 – 4.65 mg/L, 25 - 26°C, 0.25 – 1.00 ppm, 0 – 0.5 ppm and 0 – 5.00 ppm respectively. These results showed that the different feeding regimes did not affect the water quality in the experimental tanks.

Thyroxine

Fry of *P. tweediei* were immersed with thyroxine (T_4) at dosages of 0, 0.5, 1.0 ppm (Table 6). Initial total length TL of fry was 7.9mm. At 56 DAH, highest (p<0.05) TL of fry was observed after immersion with 0.5 ppm of T_4, followed by 1.0ppm T_4, and the lowest (p<0.05) in 0 ppm. Similar results were observed at 84 DAH. At the end of the experimental period, fry were able to achieve TL of 30.49 mm after immersion of 0.5 ppm of T_4. As for survival, no significant different (p>0.05) was observed for fry immersed among the three dosages of T_4 at 56 and 84 DAH. These results showed T_4 at 0.5 ppm improved the growth, and most importantly no adverse effect on survival of *P. tweediei* fry.

Table 6: Mean of total length (TL) and survival of *Parosphromenus tweediei* fry for 56 days experimental period after different dosages of thyroxine immersion

Day after hatch (DAH)	Parameters	Thyroxine (ppm)		
		0	0.5	1.0
28	Mean initial TL (mm)	7.90 ± 0.02[a]	7.90 ± 0.03[a]	7.90 ± 0.05[a]
56	Mean TL (mm)	20.60 ± 0.08[a]	21.49 ± 0.05[c]	21.01 ± 0.05[b]
	Survival (%)	100.00 ± 0.00[a]	90.0 ± 0.31[a]	90.0 ± 0.31[a]
84	Mean TL (mm)	29.73 ± 0.08[a]	30.49 ± 0.10[c]	29.99 ± 0.06[b]
	Survival (%)	100.00 ± 0.00[a]	90.0 ± 0.31[a]	90.0 ± 0.31[a]

Mean values with the same superscripts within the same row are not significantly different (p>0.05); ±SD.

Mean values for DO, temperature, ammonia, nitrite, nitrate were not significantly different (p>0.05) between 0, 0.5 and 1.0 ppm of T_4 treatments (Table 7). Ranges of DO, water temperature, ammonia, nitrite and nitrate during the experimental period were 3.85 – 4.18 mg/L, 25.3 – 26.3°C, 0– 0.5 ppm, 0 – 0.25 ppm and 0 – 5.00 ppm respectively. These water quality results showed no significant different (p<0.05) between the different T_4 dosages for all the water parameters measured, thus provided evident that T_4 is solely responsible for the significant growth of *P. tweediei* fry.

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Table 7: Water quality parameters in culture tank of *P. tweediei* fry with different thyroxine dosage throughout the 56 days experimental period

Water Parameter	Thyroxine (ppm)		
	0	**0.5**	**1.0**
Dissolved oxygen (mg/L)	3.89 ± 0.02[a]	4.10 ± 0.01[a]	3.91 ± 0.03[a]
Range	3.85 - 4.18	3.98 - 4.15	3.86 - 4.11
Temperature (°C)	25.53 ± 0.04[a]	25.61 ± 0.02[a]	25.54 ± 0.05[a]
Range	25.4 - 26.3	25.3 - 26.0	25.4 - 26.3
Ammonia (ppm)	0.41 ± 0.03[a]	0.45 ± 0.01[a]	0.41 ± 0.02[a]
Range	0.00 - 0.50	0.00 - 0.50	0.00 - 0.50
Nitrite (ppm)	0.20 ± 0.01[a]	0.24 ± 0.02[a]	0.20 ± 0.01[a]
Range	0.00 - 0.25	0.00 - 0.25	0.00 - 0.25
Nitrate (ppm)	4.76 ± 0.04[a]	4.76 ± 0.05[a]	4.72 ± 0.01[a]
Range	0.00 – 5.00	0.00 – 5.00	0.00 – 5.00

Mean values with the same superscripts within the same row are not significantly different (p>0.05); ± SD.

Discussion

Hydrogen ion concentration is reflected as pH in water. It plays an important role for aquatic animals particularly fish (Miron *et al*., 2008). It affects the normal physiological function of aquatic organism, whereby the increase or decrease will disrupts respiration, ion exchange and ammonia excretion (Schlenk and Benson, 2001; Mahassen *et al*., 2011). For freshwater fish, pH is a major determinant for certain species because the tolerance limit varies depending on species (West *et al*., 1997; Mills *et al*., 2000; Greig *et al*., 2010). Most inland freshwaters inhabited by fish have water pH range of 6.0 to 9.0 (Ellis, 1937). Nevertheless, there are fishes living in water with pH of 4.0 and 10.0 (ORVWSC, 1955). In the present study, *P. tweediei* broodstock were sampled from natural habitat at Pontian, Johor, with pH range of 4.3 to 5.9. This result showed that this fish can tolerate acidic water condition. Even Kottelat and Ng (2005) reported *P. tweediei* lives in water with pH 4.0.

According to Boyd (1982), growth and reproduction of aquatic species will decrease as pH lowered to less than 6.5 for a prolonged period. In a gourami species, *Trichogaster lalius*, exposure to pH 5.7 will cause tremendous stress and even mortality (Sachin and Subhendu, 2018). In the present study, findings from pH experiment clearly showed that not only *P. tweediei* are able to tolerate low pH, but also grow and survive best in pH 4.5 to 5.0. At pH 5.5 and 6.0, growth and survival of *P. tweediei* are adversely affected, possibly due to the decreased of acid-base balance in species with preference towards acidic condition. Wilkie and wood (1991) observed that if the pH is beyond tolerable range, fish will die due to the inhibition of ammonia excretion through the gills. Nonetheless, pH tolerance in fish will also depend on various other factors such temperature and DO (Mckee and Wolf, 1963).

In addition to the limited availability of live food, prey preferences further aggravate the condition which may affects growth and survival of fish fry

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(Pham, 2001). It is quite common that type and size of prey selected by fry change during larval developmental stage. In most cases, fish fry generally preferred small size prey (Srikrishan *et al*., 2017). The abundance of small zooplankton influences feeding success (Hansen and Wahl, 1981). However, bigger prey is preferred as fry grew larger and the size of mouth becomes wider (Gill and Hart, 1996). Therefore, Laron (2001) suggested the combination of small and large live food to provide good results. In the present study, feeding regime AMG2 utilizes *Artemia* nauplii for fry from 6 to 20 DAH, then *Moina* sp. for fry from 27 to 48 DAH, and finally grindal worm for fry from 49 to 63 DAH, produced the best growth of fry from 28 to 63 DAH. *Artemia* nauplii were given for a longer time in AMG2. According to Stickney (1994), *Artemia* nauplii is a must include food in general feeding strategy for fish fry. Srikrishan *et al*. (2017) reported that feeding with *Artemia* nauplii resulted in higher survival as compared to *Moina* sp. Consequently, the longer used of *Artemia* nauplii as initial food, the higher the survival rate of fry.

Laron (2001) suggested the combination of small and large live food to improve growth and survival catfish fry. In the present study, the inclusion of grindal worms, *Enchytraeus buchholzi* in AMG1 and AMG2 resulted in better growth of *P. tweediei* fry as compared to without it in AM. Grindal worm is being used in aquaculture due to their nutritional characteristics, availability, abundance, soft body, motile and short life cycle (Luna-Figueroa *et al*., 2000; Erdogan and Olmez, 2009). As live food, this worm is able to contribute 11.6% of protein (Bouguenec, 1992). The effect on growth of *Pterophyllum scalare* fry fed with grindal worm is equivalent to that of artificial feed (Luna-Figueroa *et al*., 2000) but lesser than *Moina*, decapsulated *Artemia* and *Daphnia* (Sorianao and Hernendez, 2002, Ortega-Salas *et al*., 2009; Santillan, 2011; Jimenez-Rojas *et al*., 2012). This is basically due to other live foods having comparatively higher protein content, better distribution and longer availability in the water column, and at the same time these characteristics reduce the creation of dominant fish during feeding (Sorianao and Hernendez, 2002; Luna-Figueroa *et al*., 2010; Takahashi *et al*., 2010). Mosquito larvae and blood worm which are larger than grindal worm can also be considered for *P. tweediei* fry from 44 DAH onwards.

Various studies have shown the growth promoting effect of thyroxine (T_4) to fish larvae (Nacario, 1983; Lam and Sharma, 1985; Reddy and Lam, 1992; Woodhead, 1996). In the present study, immersion of T_4 at 0.5 and 1.00ppm resulted in significantly accelerated growth of *P. tweediei* fry. The half-life of T_4 is 5-7 days, where it will convert into Tri-iodothyronine (T_3) or eliminated from the system (Barac-Latac, 2009). Study by Nacario (1983) showed that lower dosage T_4 increases the length of the pectoral fin significantly, while at higher dosage it

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resulted in the pectoral fin malformation and scoliosis. Overall, the growth performance of fish may differ depends on its sensitivity at different stages (Lam *et al*., 1985). In the present study, scoliosis was observed on some of the *P. tweediei* fry immersed with 1 ppm T_4. Hence, it is highly recommended to use the lower dosage of 0.5ppm T_4 immersion for *P. tweediei* fry.

In the present study, *P. tweediei* fry are able to tolerate DO as low as 3.05ppm, while temperature as low as 25 °C. As for ammonia, nitrite and nitrate, *P. tweediei* fry are able tolerate as high as 1, 1 and 5 ppm, respectively. Ammonia level is influenced by temperature and pH. As water temperature rises ammonia become more toxic (Levit, 2010). However, the temperatures recorded in all the experiments in the present study were quite low, ranged from 25 to 27°C. As for pH, growth of nitrifying bacteria is inhibited at low pH, thus the slow conversion of the ammonia to nitrite and nitrates. Nitrification process is inhibited at pH below 5.8 (Princic *et al*., 1998). As consequences, ammonia will be accumulated in the water and to certain level become toxic to fish. In addition, the accumulation of nitrite or nitric oxide can occur when DO level is below 2 mg/L (Goreau *et. al*., 1980; Painter, 1986). However, at low pH, ionized ammonia (NH_4^+) become less toxic as compared to higher pH with un-ionized ammonia (NH_3) (Wurts, 2003). This explained the high ammonia levels at pH 4.5. Even so, *P. tweediei* fry somehow showed the best growth and survival at this low pH condition. Nitrate highest level in this study was at 5ppm, however, this level is considered as non-toxic to fish (Francis-Floyd *et al*., 2009).

Based on the findings of this study, *P. tweediei* fry can be successfully cultured in water with pH 4.5 to 6.0. The observation on early developmental stages showed that this species can reach total length of 18.93 mm at 63 DAH. *Parosphromenus tweediei* is able to tolerate acidic water condition and higher ammonia levels, thus a considerably hardy fish. During fry to juvenile stage, it can be fed using combination of live food consisting *Artemia* nauplii, *Moina* sp. and grindal worm. Application of thyroxine (T_4) at 0.5 ppm per one time immersion at early stage can significantly promote the growth of *P. tweediei* fry. Cost of production can be reduced with the application of T_4 as the hormone will accelerate the growth of fry thus shorten the culture period.

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