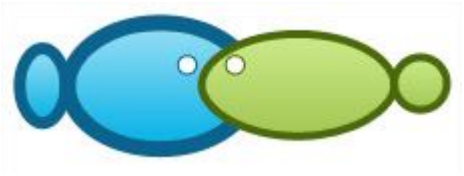

The effects of delayed initial feeding on the growth and survival of silver perch, *Bidyanus bidyanus* (Mitchell, 1838)

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Abstract. Three sets of experiments were conducted to determine the point of no return (PNR), growth rate, and survival of silver perch (*Bidyanus bidyanus*) larvae. In each of the experiments, the larvae were exposed to different initial feeding delays, starting from yolk sac exhaustion at 4 days post hatching (dph). The PNR experiment was conducted for eight days to test different delayed initial feeding of 0, 1, 2, 3, 4, 5, and 6 days respectively, while growth and survival experiments were conducted for 20 days at 0, 1, 2, 3, and 4 delayed initial feedings, with unfed larvae as a control group. The result showed that the onset of feeding occurred at 5 dph and PNR was detected between 8 and 9 dph. The specific growth rates at 0, 1, and 2 days of delayed initial feeding were significantly higher ($p < 0.05$) than the other treatments. The initial feeding also affect the survival rate where the delay of 0 and 1 day were significantly higher ($p < 0.05$) than the other treatments. These results indicate that initial feeding at 5 and 6 dph has an advantage in growth rate over initial feeding at 7 and 8 dph. Because larval survival at 5 dph initial feeding was higher than at 6 dph, the initial feeding of silver perch larvae should start at 5 dph.

Key Words: point of no return, exogenous feeding, yolk utilisation, growth, survival, silver perch.

Introduction. Some fish species have been reported as being more prone to mass mortality in their early life phase than in the advanced developing phase (Dou et al 2005). Their larval growth and survival are highly dependent on the early-life-history stage, which is extremely critical, especially during the period of transition from the use of endogenous nutritional reserves to exogenous food consumption. The delay in initial feeding after endogenous reserves are exhausted often causes morphological deformities, abnormal swimming behaviour, and an inability to catch prey (Gisbert & Williot 1997; Qin et al 1997; Mookerji & Ramakrishnan Rao 1999), leading to high mortality rates (Kailasam et al 2007; Dou et al 2005).

The availability of the right food at the right time is crucial as starvation can lead to high mortality (Dou et al 2002). It has also been described that low larval survival commonly occurred during the initial feeding periods (Bisbal & Bengtson 1995; Yang 2007), which is most affected by a delay in the initial feeding (Dou et al 2000; Gisbert et al 2004; Peña & Dumas 2005). Related to this critical part of aquaculture, the idea of the point of no return (PNR) has been applied to study the effect of starvation on fish larvae mortality (Peña & Dumas 2005; Kailasam et al 2007; Shan et al 2008; Jinbo et al 2013). The PNR is interpreted as the stage where the effects of food shortage become irremediable. Hence, about 50% of starving larvae remain alive, but are powerless to feed even if food is available, thus they cannot pass the ontogenetic stage successfully. The duration between yolk sac exhaustion and the PNR is specific depending on the fish species (Shan et al 2008), but there is currently no information available for silver perch, *Bidyanus bidyanus*.

Silver perch is a freshwater fish species endemic to the Murray-Darling river system in New South Wales, Australia, and is also known as freshwater bream, silver

bream, and grunter. Silver perch belongs to the Terapontidae family, and occupies the northern and western rivers and upper reaches of the Murray-Darling river system (Rowland 1995). Silver perch has also been introduced in different parts of the world, for example, in Israel in 1997 (Moiseeva et al 2001), and in Taiwan in the early 1990s (Yang et al 2006; Yang et al 2011).

The newly hatched silver perch larvae are fragile and weak swimmers, especially during the first three days (Thurstan & Rowland 1995). At this stage, the larvae are light sensitive (Gehrke 1994), and thus light intensity should be kept to a minimum (Thurstan & Rowland 1995). Not only is the availability of high-quality feed vital, but feeding technique is also important for the species being cultured (Twibell et al 2009). Failure to provide the initial feed results in a lowered survival rate (Rowland 2009). Due to the lack of knowledge regarding the behaviour of the silver perch larvae, feeding techniques have generally been developed using trial and error (Phipps 1999).

The use of earthen ponds for larval production is popular due to their practicality, reliability, and commercial viability (Ogburn et al 1995). Considerable problems have been identified, however, such as intensive labour, bird predation (Barlow 1995), and vulnerabilities related to exposure to disease organisms, such as the frequently encountered ectoparasitic protozoan, *Trichodina* sp. (Thurstan & Rowland 1995), which causes trichodiniasis (Callinan & Rowland 1995). Due to the disadvantages associated with pond rearing of silver perch larvae, aquaculturists are looking to develop hatchery rearing techniques. Hatchery production of silver perch larvae is possible, but feed and feeding techniques need to be improved (Phipps 1999).

The description of how an initial feeding delay affects the fish larvae conditions under specific culture systems can be a useful tool for assessing the capability of the hatchery settings, and could aid the discovery of ways to prevent death triggered by hunger. The aim of this research was to determine the occurrence of the PNR, and investigate the larval growth and survival performance of silver perch larvae in laboratory conditions after progressive starvation by an initial feeding delay.

Material and Method. The three experiments and the procedure in this study were approved by the Animal Ethics Committee of Curtin University (approval number AEC_2011_70), and the Australian Code of Practice for the care and use of animals for scientific purposes is followed.

Experimental larvae. The fish larvae used in this experiment were produced from the domesticated silver perch broodstock that has been reared at the Curtin Aquaculture Research Laboratory (CARL) for around five years. The human chorionic gonadotropin (hCG) hormone was injected into both female (weight 3.5 kg) and male (weight 1.6 kg) broodstock at a dosage of 200 IU kg^{-1} of fish to obtain eggs (Rowland 1984; Levavi-Sivan et al 2004; Rowland 2009). After the hormone injections, paired broodstock were kept in the hatchery within the same tank at a room temperature of 20-26 °C until they spawned. After spawning, around 20,000 eggs were transferred to two 200 L conical shape fiberglass incubator tanks filled with filtered fresh water equipped with an aeration from the bottom. The time at which 90% of the viable eggs were hatched was defined as the hatching time (Shan et al 2008), with a hatching rate of 86.5%. The larval density in the incubation tanks was then estimated with a volumetric system by taking triplicate 10 mL sub-samples (Jensen et al 2013), which were then stocked into a 200 L cylindrical holding tank at a density of 100 ind L^{-1}. The holding tank was equipped with gentle aeration and the temperature was maintained at 23°C. The larvae in this tank were kept unfed until the end of the experiment as a stock larvae for PNR determination, growth experimentation, and survival examination.

The experimental chambers used for the PNR determination were 1 L glass beakers and 20 L plastic tanks, which were used for the growth and mortality experiments. Rotifers, *Brachionus calyciflorus* was used as food for the larvae. This was harvested from laboratory culture that had been maintained on green alga *Chlorella* sp. At a chosen time of initial feeding for each treatment, the larvae were delivered rotifers at a density of around 10 ind mL^{-1}. Aeration was provided to encourage a homogeneous

distribution of live food, and also to maintain the dissolved oxygen levels. Prey density was checked daily before feeding to maintain them at the desired level. The water quality parameters, including temperature, pH, and ammonia, were measured and recorded daily. Temperature was recorded by a temperature data logger (Onset HOBO Data Loggers, made in USA), pH was measured with a pH meter (Cyberscan pH 300, Eutech Instruments, Singapore), and total ammonia was measured with chemical test kits (Aquarium pharmaceutical Inc., Chalfont, PA, USA).

PNR determination. Seven treatments, each of with three replications of initial feeding were tested from 4 to 11 dph. At 24 h intervals, 20 larvae were randomly taken from the holding tank and moved into 1-L glass beakers. The beakers were placed in an incubator tank equipped with thermostatic control (Thermomix, B. Braun Biotech International) to maintain the water temperature at around 23^{o}C. A temperature data logger (Onset HOBO Data Loggers) was placed in the incubator tank to record the temperature, and 40W fluorescent light regulated at around 200 lux was used to accommodate the visual feeding behaviour of silver perch (Phipps 1999; Thurstan & Rowland 1995). In addition, every beaker was provided with individual mild aeration. The swimming and feeding behaviour of the larvae was then visually observed, and the time when the larvae indicated signs of starvation, such as sluggish swimming behaviours and hanging their heads down in the water column, were monitored during 4 h of feeding time.

In order to recognise the presence of prey in their guts, larvae were first placed in the 1-L glass beaker contained rotifers at a density of 10 ind mL^{-1}. After the larvae were exposed to rotifers for 4 h (Dou et al 2005), all larvae were observed under a stereo microscope to count the number of rotifers in the gut (Wang et al 2010). This was used to calculate the feeding rate and feeding intensity. The feeding rate, as defined by Dou et al (2005), is the proportion of larvae that are able to catch food after progressive food shortages imposed on the total number of stocked larvae. The feeding intensity is the number of prey in the gut at dissection. The time when the feeding rate of progressively starved larvae is lower than 50% of the maximum feeding rate when food was supplied is defined as the PNR (Dou et al 2005). The experiment was continued until all larvae died, or the starving larvae could no longer initiate feeding.

Effect of delayed initial feeding on larval development and growth. Six treatments were applied in triplicate in this experiment. The delayed initial feeding treatment of 1, 2, 3, 4, and 5 d, respectively, was assigned to the first five treatments, while the sixth treatment involved unfed larvae assigned as a control group. At 2 dph, the larvae were randomly taken out from the holding tank and were placed in 20 L experimental tanks at 20 ind L^{-1} of stocking density. The larvae were fed on rotifers at a density of 10 ind mL^{-1}. The density of rotifers was maintained throughout the experiment via daily adjustments. From the beginning of the experiment, 10 larvae were periodically and randomly sampled from each experimental unit for larval development and growth examination. The sample was taken every four days at 12:00 during the experiment, and anesthetised with AQUI-S at 1.0 ppm before being preserved in a 10% formalin buffer and stored at 4°C. The control treatment lasted up until 11 dph as all larvae died within this period. The other treatments terminated at 20 dph, however.

Four body measurements were used to identify the effects of food deficiency on the general body morphology, i.e. total length (TL), eye diameter (ED), head depth (HD), and mouth opening (MO) (Figure 1). All measurements are related to larval quality and are commonly used to differentiate larval performance between treatments (Koumoundouros et al 1995; Gisbert et al 2002). They were measured vertically or horizontally lateral to the body axis (Gisbert et al 2004; Kailasam et al 2007). Measurements were performed to the accuracy of 0.1 mm using a stereoscopic microscope equipped with an Olympus SC30 camera that had image acquisition software getIT from Olympus Soft Imaging Solutions GmbH.

The morphometric data were processed to examine the effects of late initial feeding on larval growth rate. The specific growth rate (SGR) of the TL at different treatments was calculated by the formula: $SGR = (e^{g} - 1) \times 100\%$ (Hansen et al 2001), where $g = [\ln(l_2) - \ln(l_1)] / (t_2 - t_1)$, and l_2 and l_1 are the mean TL on days t_2 and t_1,

respectively. The initial size differences between treatments were minimised so that the average could represent the initial length (L_0) of the individuals at 4 dph. A sample of 10 larvae was taken from the holding tank for the L_0 measurement just before the initial food was offered. The coefficients of variation (CV) of TL were calculated within each treatment with the formula CV = 100 × SD/mean TL (SD = standard deviation of mean TL) to find out the effects of delayed initial feeding on the individual growth variation of the larva.

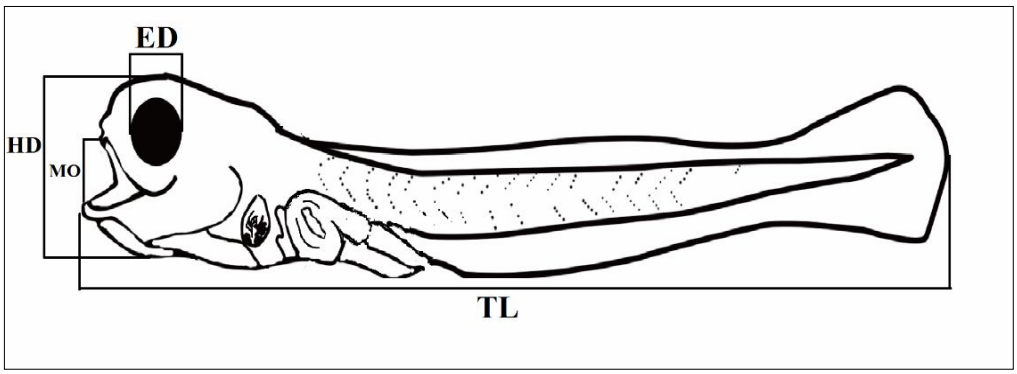

Figure 1. Morphometric measurements of silver perch larvae. ED, eye diameter; HD, head depth; TL, total length, and MO, mouth opening.

The influence of delayed initial feeding on larval survival. The third experiment investigated the changes in the survival rate of silver perch larvae exposed to different initial feeding treatments. Silver perch larvae were reared in 20 L plastic tanks at a stocking density of 20 ind L^{-1}, and initial feeding was started at 4, 5, 6, 7, and 8 dph. The feeding practice in Experiment 2 was also applied to this experiment. The experiment was conducted in triplicates and lasted after 20 dph. The experimental tanks were siphoned once every day, just before the rotifers' density was adjusted to the desired density (10 ind mL^{-1}). At the same time, the dead larvae were removed from the tank through siphoning. Based on the daily count of dead larvae, the survival was calculated as the percentage of the alive fish in the tank.

Data analysis. The data analysis was performed using the IBM SPSS-24 software package, and a significance level of $p < 0.05$ was used. One-way ANOVAs, followed by Tukey tests when appropriate, were used to evaluate the differences in the means of the morphometric variables, including TL, HD, ED, MO, and survival rate at different delayed initial feeding times. The data in percentage were arcsine transformed prior to statistical analysis. Results are shown as the mean±standard error of the mean (SE).

Results

PNR determination. The eggs were hatched after 35 h of incubation at 21±1°C. The newly hatched larvae had a slim and elongated shape with a large yolk sac (0.32±0.02 mm^3) containing an oil globule on the posterior tip, which had drifted frontally as the yolk sac reduced. Pigmentation appeared as small dots scattered around the body and yolk sac. No eye and mouth openings were observed for the 2.65 mm TL of newly hatched larvae (Figure 2a). At 2 dph, the yolk sac reduced extensively and the pigmentation of the eyes was noticed (Figure 2b). The distal region of the digestive tract was visible as a tube behind the oil globule. At 3 dph, the yolk reserves were intensively utilised, and the oil globule moved forward. The eye pigmentation appeared to have higher intensities, and the mouths started to develop (Figure 2c, inverted in colour). Yolk sac exhaustion started at 4 dph, and the remaining oil globule was visible. At this time, the larvae had developed most of their organs, such as eyes, jaws, mouths, swimming bladders, and digestive systems (Figure 2d). The onset of the first feed was noticed at 5 dph, however, while the maximum feeding rate and feeding intensity occurred at 6 dph. Around 28.3±1.7% of larvae were able to feed at 5 dph (Figure 3), which increased significantly ($p < 0.05$) to 80.0±0.3% at 6 dph when the maximum feeding rate occurred (Figure 3).

Beyond 6 dph, the feeding rates dropped down to 53.3±0.1 (higher than 50% of the maximum feeding rate) at 8 dph, and 33.0±0.1% (less than 50% of the maximum feeding rate) at 9 dph. Therefore, the PNR of silver perch was between 8 and 9 dph.

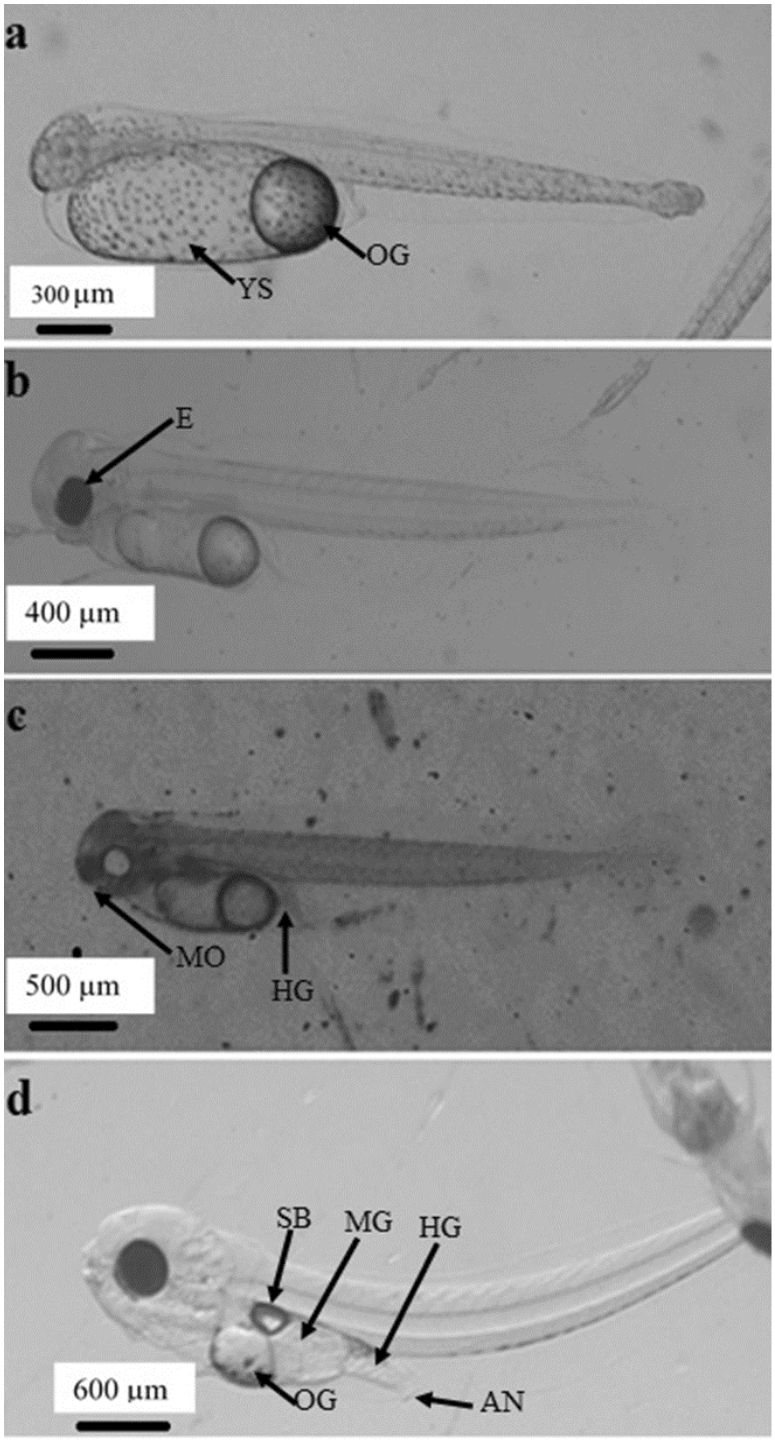

Figure 2. Larval development of silver perch (*Bidyanus bidyanus*): the newly hatched larvae (a); larvae at two dph (b); larvae at 3 dph (c); and larvae at 4 dph (d). OG = oil globule; YS =yolk sac; E = eye, MO = mouth opening; SB = swim bladder; HG = hindgut, MG = midgut; AN = anus.

This point is shown in Figure 3 where the dash line (50% of the highest feeding rate) is intercepted by the line graph of the feeding rate. The feeding rates at 6 and 7 dph showed no significant differences ($p > 0.05$). The symptoms of starvation were noticed at 8 dph, and most larvae were starved irreversibly at 9 dph. The feeding rate decreased to only 10% at 10 dph, at which time most larvae were dead. The pH, dissolved oxygen, and ammonia were found to be within the accepted ranges of 7.51±0.01, 7.45±0.01 mg

L^{-1}, and 0.00±0.00 mg L^{-1}, respectively, over the experimental period (Rowland 2009; Frances et al 2000).

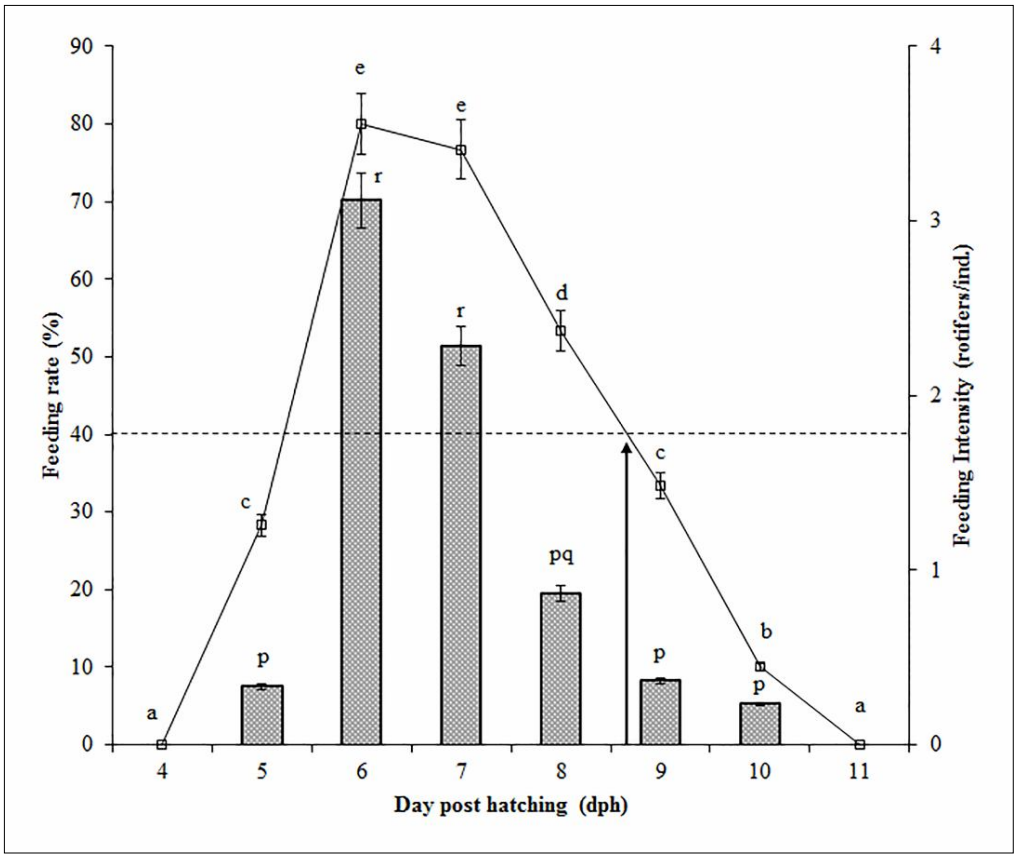

Figure 3. Changes in the initial feeding rate (line graph) and feeding intensity (bar graph) of starved silver perch larvae when firstly presented with food from 4 to 11 dph, the intersection of feeding rate curve and broken line signals the PNR (↑). Points sharing different lower case letters in the same dph for each graph indicated significant differences between treatments (ANOVA, $p < 0.05$).

Effect of delayed feeding on larvae growth rate. The growth rate of silver perch larvae was strongly affected by delayed initial feeding. Starved larvae could survive until 10 dph, but the different initial feeding treatments lingered until the end of the experiment at 20 dph. Therefore, the relationship between delayed initial feeding and larval growth rate at a period of 4-12 dph and 12-20 dph were investigated at 12 and 20 dph, respectively (Table 1). The TL and the SGR showed significant differences ($p < 0.05$) at both 12 dph and 20 dph for different initial feeding times. The growth rates of 5 and 6 dph initial feeding larvae (SGR = 5.19±0.63, TL = 6.45±0.25 mm, and SGR = 4.85±0.53, TL = 6.27±0.29 mm, respectively) were not statistically different ($p > 0.05$), but they were significantly higher ($p < 0.05$) than unfed larvae (SGR = 1.23±0.36, TL = 5.11±0.39 mm) at 12 dph. The growth rate of 6 dph initial feeding larvae was also significantly higher than the 8 dph initial feeding larvae (SGR = 1.12±0.37, TL = 4.99±0.38 mm) at 12 dph (Table 1). At 20 dph, the growth rate did not show any significant differences ($p > 0.05$) between the 4, 5, and 6 dph initial feeding larvae (SGR = 5.39±0.24, TL = 9.94±0.41 mm; SGR = 5.72±0.09, TL = 10.45±0.41 mm; and SGR = 5.56±0.02, TL = 10.19±0.2 mm, respectively), but they were all significantly higher ($p < 0.05$) than the 7 and 8 dph initial feeding larvae (SGR = 4.59±0.14, TL = 8.24±0.37 mm, and SGR = 3.00±0.18, TL = 6.05±0.19 mm, respectively) (Table 1). Whether at 12 dph or at 20 dph, size variation tended to increase with a delay of initial feeding (Table 1).

Table 1

Final total length (TL, mean±SE), coefficient of variation (CV) of total length and specific growth rate of total length (SGR, mean±SE) for silver perch larvae at a different time of initial feeding

Initial feeding	*TL (mean±SE)*	*CV*	*SGR (mean±SE)*
	12 dph		
D4	5.76 ± 0.20^{a}	5.3	4.67 ± 0.32^{b}
D5	6.45 ± 0.25^{a}	6.2	5.19 ± 0.63^{b}
D6	6.27 ± 0.29^{a}	7.9	4.85 ± 0.53^{b}
D7	5.57 ± 0.26^{a}	8.1	2.97 ± 0.66^{ab}
D8	4.99 ± 0.38^{a}	13.3	1.12 ± 0.37^{a}
UF	5.11 ± 0.39^{a}	13.3	1.23 ± 0.36^{a}
	20 dph		
D4	9.94 ± 0.41^{c}	3.16	5.39 ± 0.24^{c}
D5	10.45 ± 0.2^{c}	3.32	5.72 ± 0.09^{c}
D6	10.19 ± 0.03^{c}	3.57	5.56 ± 0.02^{c}
D7	8.24 ± 0.37^{b}	7.87	4.59 ± 0.14^{b}
D8	6.05 ± 0.19^{a}	5.71	3.00 ± 0.18^{a}

Different superscript letters (^{a,b,c})in the same column at different dph indicate significant differences between treatments (ANOVA, $p < 0.05$).

The effects of delayed initial feeding time on the measured morphometric of silver perch larvae are presented in Figure 4.

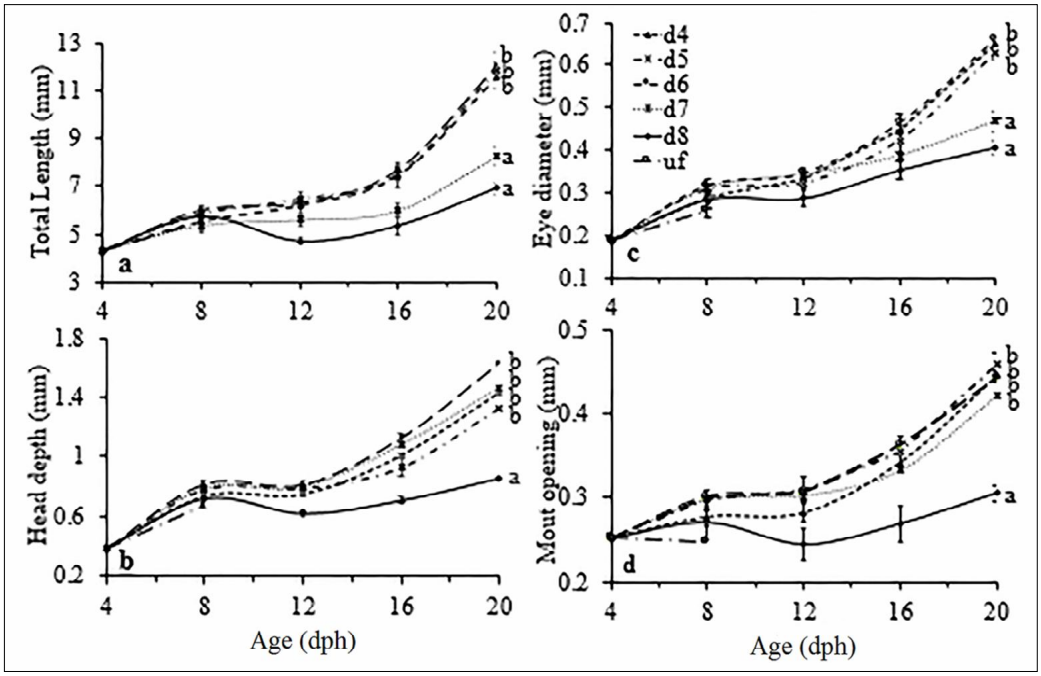

Figure 4. Morphometric changes of silver perch larvae at different initial feeding time of 4, 5, 6, 7, 8 dph and unfeed (uf) larvae. Points sharing different lowercase letters in the same age indicated significant differences between treatments (ANOVA, $p < 0.05$).

Significant differences ($p < 0.05$) for the morphometric parameters were noticed in the initial feeding larvae of 4, 5, 6, 7, and 8 dph, which represented 0, 1, 2, 3, and 4 days after yolk sac exhaustion, respectively. No significant differences in TL were identified, however, from 4 to 20 dph between the 4, 5, and 6 dph initial feeding larvae. On the other hand, when the larvae were starved for 3 and 4 days (7 and 8 dph initial feeding), the TL was significantly smaller ($p < 0.05$) than the 4, 5, and 6 dph initial feeding larvae at 12, 16, and 20 dph (Figure 4a). The TL of the 4, 5, 6, and 7 dph initial feeding larvae gradually grew between 4 and 16 dph ($p > 0.05$) before rapidly growing at 20 dph ($p < 0.05$). The 8 dph initial feeding treatment showed fluctuating growth patterns between 4

and 20 dph. Changes in HD, ED, and MG all followed a similar pattern to the TL development with the 4, 5, and 6 dph initial feeding larvae being significantly higher in these measurements ($p < 0.05$) than the 7 and 8 dph initial feeding larvae at 12, 16, and 20 dph (Figures 4b, 4c, and 4d, respectively). The pH, dissolved oxygen, and ammonia were found to be within the accepted ranges of 7.50±0.01, 7.44±0.01 mg L^{-1}, and 0.00±0.00 mg L^{-1}, respectively, over the experimental period (Rowland 2009; Frances et al 2000).

Effect of initial feeding on survival rate. The survival rate of silver perch was affected by different delays in initial feeding (Figure 5). After 20 days of the rearing period, the highest survival rate of 50±2.3% was noted for the 5 dph initial feeding larvae, which was not significantly different ($p > 0.05$) to the survival rate of the 4 dph initial feeding larvae. A lower final survival rate was observed when the larvae were exposed to an initial feeding delay of 6, 7, and 8 dph (39%, 20%, and 8%, respectively). Whole mortality of unfed larvae was noted on 10 dph. The survival rate decreased significantly ($p < 0.05$) from 84% in the 5 dph initial feeding larvae, to 57% in the unfed larvae at 8 dph (Figure 5). The 3 and 4 days delayed initial feeding and unfed larvae had the same survival trends, dropping down rapidly from 5 dph to 8 dph (Figure 5). The water quality in all experimental units, including pH, dissolved oxygen, and ammonia, was within the range for optimum growth of silver perch (7.50±0.01, 7.44±0.01 mg L^{-1}, and 0.00±0.00 mg L^{-1}, respectively) over the experimental period (Rowland 2009; Frances et al 2000).

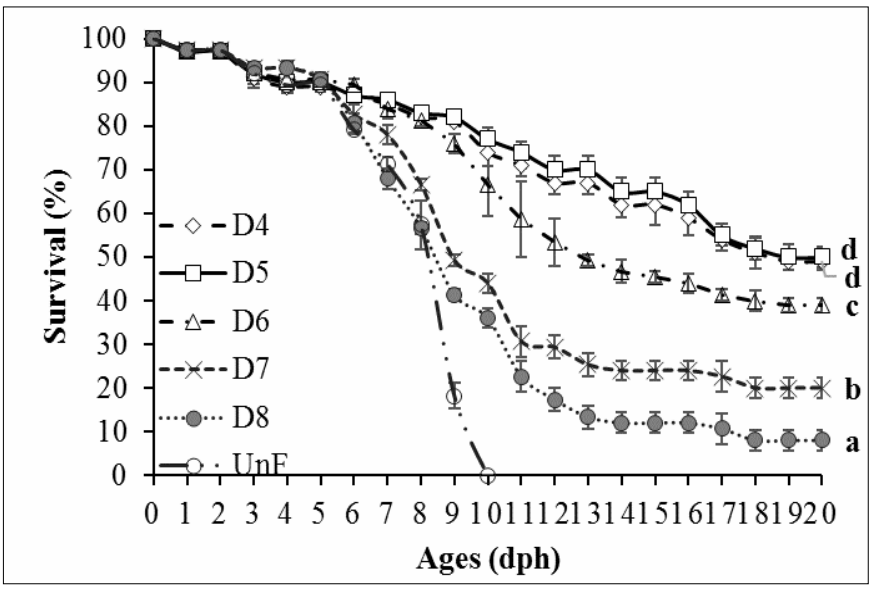

Figure 5. Survival rate of silver perch larvae from 0 to 20 dph at different delayed initial feeding. Different lower case letters in the same column indicated significant differences between treatments (ANOVA, $p < 0.05$).

Discussion. The commencement of the initial feeding of fish larvae varies depending on the species (Dou et al 2005; Yúfera & Darias 2007). Silver perch larvae initiating feeding exogenously at 5 dph was consistent with our previous experiment (unpublished data). The onset of feeding on rotifers was not related to MO, as larvae opened their buccopharynx two days before the first feeding was noticed (3 dph). In the current study, the initial feeding of silver perch corresponded with the change of the larva's behaviour from passive to actively swimming, and from sinking to the bottom to maintaining their position in the water column. According to Kamler (1992), these kinds of changes in fish activity are commonly associated with the depletion of endogenous food and the onset of exogenous feeding. Hence, alterations in the dispersal activities of fish larvae in rearing tanks are often used as visual criteria to start feeding (Shan et al 2008). Compared to other fresh water fish larvae, silver perch larvae start feeding at a moderate time (5 dph). Some species start earlier (2-4 dph), while others start later (6-9 dph), as summarised in Table 2.

Table 2

Average temperature (T), time from hatching to first feeding (tf), time from hatching to yolk absorption (yolk sac + oil globule) (ty), time from hatching to PNR (PNR), and time from yolk absorption to PNR (ty-PNR) of some fresh water fish

Species	*T (^oC)*	*tf (d)*	*ty (d)*	*PNR (d)*	*Ty-PNR (d)*	*Sources*
Tilapia, *Oreochromis mossambicus*	28.0	6.0-7.0	15.0	16.0-19.5	1.0-4.5	Rana (1985)
Singhi, *Heteropneustes fossilis*	26	3.0	6.0	8.0	2.0	Mookerji & Ramakrishnan Rao (1999)
Rohu, *Labeo rohita*	26.0	2.0	9.0	14.0	5.0	Mookerji & Ramakrishnan Rao (1999)
Carp, *Aspidoparia morar*	14.0	4	9.0	14.0	5.0	Malhotra & Munshi (1985)
Siberian sturgeon, *Acipenser baeri*	18	9	10	-	-	Gisbert & Williot (1997)
Chinese sturgeon, *Acipenser sinensis*	22-24	8	10	15	5	Chai et al (2011)
Loach, *Misgurnus anguillicaudatus*	23	3	6	9-10	3-4	Wang et al (2010)
Carp, *Aspidoparia morar*	13-15	7	9	14	5	Malhotra & Munshi (1985)
Burbot, *Lota lota*	12	5	14	10	5	Palińska-Żarska et al (2014)
Silver perch, *Bidyanus bidyanus*	21	5	10	8-9	4-5	This study

The duration from yolk depletion to PNR is different from one species to another. In this research, the PNR of silver perch was detected between 8 and 9 dph, or about 4-5 days after the yolk-sac was depleted. This period in other fish species varies from less than one day in sea bream, *Archosargus rhomboidalis*, and lined sole, *Achirus lineatus*, (Houde 1974), 6 days in silver catfish, *Rhamdia voulezi* (de Lima et al 2017) to 6 weeks in Atlantic salmon, *Salmo salar* (Koss & Bromage 1990; Peterson & Martin-Robichaud 1995). Hence, the ability of fish to survive under food shortage conditions is also dependent on the eggs and yolk sac sizes (Gisbert et al 2000). Large eggs are likely to carry more nutrients, resulting in a bigger yolk sac, which allows a longer time to before initial feeding, and lengthens the time required to reach the PNR. This means that the PNR of larger eggs will be relatively longer than smaller eggs. Compared to species with a large egg size, such as Siberian sturgeon, *Acipenser baeri* (2.8-4.1 mm), and species with a strong starvation resistance (Gisbert et al 2000), the silver perch larvae in this study had a lower egg size (averaging 2.2 mm).

The initial feeding of fish larvae tends to relate to the development and functionality of different body parts, such as sensory, buccal, and swimming organs. The late development of these organs often causes initial feeding rates in most fish larvae at lower a range of 10-50% (Shan et al 2008). Based on the onset of feeding and the ability to survive during periods of food shortage, silver perch larvae belong to type 'A', where the feeding rate at the initial feeding is low, before sharply improving, then continuing to rapidly decline (Shan et al 2008). At the beginning, the feeding rate was 28%, which then increased to the highest feeding rate of 80% at 6 dph, and decreased to 35% at 9 dph. The initial feeding time occurred when the swimming capability of the silver perch larvae was still poor, and some structures connected to food digestion had not yet become fully functional. This was in agreement with the findings regarding other fish species (Dou et al 2005). On the other hand, the number of larvae involved in feeding activities increased with starvation until 6 dph, indicating that the desire for food enhanced to recompense food constraint during starvation. After 7 dph onward, however, both feeding intensity and feeding rate experienced a significant decrease ($p < 0.05$). This may indicate that the digestive system of silver perch can rapidly decline when exposed to starvation. The empty guts of the larvae showed incomplete development with weak pigmentation, and were thus transparent in colour. The same phenomenon was observed by Shan et al (2008) in sea bream, where the development of their digestive tracts stops and the intestine becomes pale after starvation.

The increase in the length of silver perch larvae was significantly affected by the delayed initial feeding. Even if the mean size of the larvae displayed an increase at a shorter delay of initial feeding, it caused negative growth at a longer delay of initial feeding. The negative effect of delayed initial feeding was clearly shown in TL and SGR. When the initial feeding was delayed for 4 days, the TL and SGR of the silver perch larvae were significantly lower than at 0 and 1 day initial feeding delays at 12 and 20 dph. Hence, negative growth was most likely related to the fact that larvae in this treatment could not procure exogenous feeding. On the other hand, the TL and SGR did not differ significantly between un-delayed initial feeding larvae and 1 day delayed initial feeding larvae. The negative effects on larval growth after the delayed initial feeding (the time threshold) were observed at 2 days before the PNR at 23°C. The delayed initial feeding of the silver perch larvae also led to an increase in TL variation. The longer the delay of initial feeding, the larger the CV values observed. The same condition has also been observed in other fish, such as Chinese sturgeon, *Acipenser sinensis* (Chai et al 2011), summer flounder, *Paralichthys dentatus* (Bisbal & Bengtson 1995), and rock bream, *Oplegnathus fasciatus* (Shan et al 2008). The size variation here is probably promoted by the differences in individual feeding ability and the behaviour of the larvae after being exposed to different initial feeding delays. The energy needed to maintain life and the allocated energy for growth as well as for feeding activity could vary greatly, resulting in high individual variability in size. The longer the initial feeding delay, the more energy spent on the life maintenance of the starving larvae, and the higher the individual variation.

The delayed initial feeding was not only important in influencing the growth performance of the larvae, but also contributed significantly to the survival rate (Houde 1974). The best survival rate at the end of this research was observed for un-delayed initial feeding, and at initial feeding delayed by 1 day, which was higher compared to the survival rates at 2, 3, and 4 days. All larvae were dead at 6 days of delayed feeding at 10 dph. This was comparable to another freshwater fish, the loach, *Misgurnus anguillicaudatus,* where the survival rate at 3 dph initial feeding was higher than at initial feeding of 4, 5, and 6 dph (Wang et al 2010). The delayed initial feeding approaching the PNR may alter the feeding ability more seriously, which is generally accompanied by high mortality as a result of decreased growth (Abi-ayad et al 2000). Hence, the reduction in larval sizes is conclusive evidence that starved survivors have done so at the expense of tissue formation (Dou et al 2005). Even if whole larval death was noticed at 10 dph in this study, the growth and survival rates were very poor for the 7 and 8 dph initial feeding larvae. Based on the survival and growth rate performance of the silver perch larvae, it is suggested that a delay of initial feeding can be tolerated for 2 days, from 4 to 6 dph.

Conclusions. The beginning of exogenous feeding in the silver perch larvae occurred at 5 dph, one day later than the yolk sac exhaustion. The elapsed time from the beginning of initial feeding to the PNR was around 3 days, placing it between 8 and 9 dph. Larval growth and their survival rates were directly associated with the time of initial feeding. Findings also indicated that 5 dph initial feeding gives the best growth and survival rate performance. Therefore, an initial feeding of 5 dph is suggested for the hatchery production of silver perch larvae.

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