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REPRODUCTIVE PERFORMANCE OF DOMESTICATED BROODSTOCK OF SILVER PERCH, *Bidyanus bidyanus* (MITCHELL 1838) AND THE RELATIONSHIP BETWEEN OIL GLOBULE FRAGMENTATION AND EGG QUALITY

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ABSTRACT

The experiments investigated the reproductive performance of the domesticated broodstock of the silver perch and the relationship between various degrees of oil globule fragmentation and egg quality. Six years old of second generation broodstock (n=3) were evaluated based on the fecundity, fertilisation rate, hatching rate, the degree of oil fragmentation of egg, and the quality of embryos and larvae produced. The fragmentation were grouped into three categories: un-fragmented (cat-1), moderately fragmented (cat-2), and highly fragmented (cat-3). The results showed that the broodstock had a relatively high fecundity (132,400 ± 7,22), fertilization rate (94.27 ± 1.28%), and hatching rates (87.94 ± 1.23%). The survival rate of larvae at 12 days post hatching (dph) in cat-1 (71.3 ± 0.9%) was higher than cat-2 (66.7 ± 0.9%) whereas cat-2 was higher than cat-3 (61.3 ± 0.3%). The eggs was dominated by cat-1 (78.11 ± 2.44%) which was significantly higher than cat-2 (21.26 ± 2.45%) and cat-3 ones (0.40 ± 0.21%). The survival rate of embryo at 20 hours post spawning (hps) and hatching rate of cat-1 (95.33 ± 0.00% and 93.33 ± 0.00%) and cat-2 (90.00 ± 0.00% and 85.00 ± 0.00%) were significantly higher than cat-3 (72.33 ± 1.76% and 60.33 ± 0.00%). The total length (TL) of the larvae of cat-1 and cat-2 (8.44 ± 0.21 mm and 8.35 ± 0.23 mm respectively) were significantly higher than larvae of cat-3 (7.09 ± 0.14 mm). No significant difference was found in the larval deformities among any categories. In conclusion, the reproductive performance of six year-old broodstock silver perch showing acceptable performance and egg categorisation based on oil globule fragmentation can be used as a useful tool to indicate eggs quality of silver perch.

KEYWORDS: reproductive; oil globule; egg quality; perch; *Bidyanus*

INTRODUCTION

Silver perch, *Bidyanus bidyanus* (Mitchell, 1838) is well known as a potential native species for aquaculture in Australia inhabiting inland waters around the Murray Darling River system (Allan & Rowland, 2002; Rowland, 2004a). Silver perch has been cultivated for around two decades expecting to be the largest freshwater aquaculture industry in Australia, as declared by Rowland (2009). A number of problems related to the larval production techniques including the understanding of broodstock performance and egg quality still need to be overcome.

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Mastery of the techniques of fish reproduction is an important step as it is directly related to aquaculture productivity (Zarski *et al*., 2011a; Zarski *et al*., 2011b). Silver perch hatchery has been based on the hypophysation technique to spawn the broodstocks and pond-based larval rearing techniques (Rowland, 1984; 2009). In the hatchery environment, a broodstock can be captured from the natural environment or can be domesticated using farming techniques (Rowland, 2004a). However, Rowland (2009) stated that the performance of the wild-caught broodstock is reduced after five years of continuous spawning.

As the silver perch culture matures and farmers are more familiar with modern farming techniques of the species, some farmers are now able to produce their own larvae (Mccormack, 2017). However, various bottlenecks in pond-based larval culture are

still challenging farmers as larval quality in pond-based rearing systems cannot be controlled and/or predicted (Mosig, 2005). When domesticated broodstocks used for spawning, the use of hormone and formulated diets, and handling procedures can considerably affect the egg quality and survival of larvae (Fernández-Palacios *et al*., 1997; Almansa *et al*., 1999; Izquierdo *et al*., 2001; Watanabe & Vassallo-Agius, 2003; Targoñska *et al*., 2010). Eggs that do not develop normally, die after a while, triggering protozoan, and fungal growth that can infect surrounding embryos during incubation (Ciereszko *et al*., 2009) and then it intensifies during the egg hatching period resulting in mass mortalities due to water degradation (Rowland, 2009).

Egg quality is a complex area to quantify and is still not well understood (Jakobsen *et al*., 2016). The fatty acid composition of eggs plays an important role in improving reproduction success, including egg quality, and the survival of fry (Komen & Thorgaard, 2007; Bobe & Labbé, 2010). Egg quality of silver perch has been identified as a predominant indicator in assessing reproductive success and a key factor causing the high variation in the size of newly hatched larvae (Anonymous, 1999; Moiseeva, 2001; Rowland, 2004b; 2009).

It is widely accepted that egg quality affects both egg and larvae viability which in turn is reflected in several characteristics of larvae including physical deformity, growth rate, mortality rate, and size variability (Zarski *et al*., 2011b). The difference in individual performance can lead to high cannibalism which, at the end, will directly determine production effectiveness (Rowland, 2009). In silver perch, egg quality is commonly evaluated based on the viability of larvae after hatching (Rowland, 1984; 2009) while earlier determination of egg viability could be a beneficial tool to eliminate low quality eggs leaving incubation of only good eggs quality as in Eurasian perch, *Perca fluviatilis* (Zarski *et al*., 2011b).

The distribution of oil globules has been used as an indicator of egg quality in European perch (Zarski *et al*., 2011b) and brown trout, *Salmo truttafario* (Mansour *et al*., 2007). There was no relationship between oil globule distribution in the oocyte and the quality of the eggs especially with respect to reproductive performance of the domesticated rainbow trout (Ciereszko *et al*., 2009). However, oil globule fragmentation has never been used as a tool to evaluate the reproductive performance of silver perch. The present study was conducted to investigate any relationship between the degree of oil globule fragmentation and the reproductive performance and egg quality of the domesticated broodstock of silver perch.

MATERIALS AND METHODS

The experiments and the procedure in this study were approved by the Animal Ethics Committee of Curtin University with the approval number of AEC_2011_70 and the Australian Code of Practice for the care and the use of animals for scientific purposes also followed.

Broodstock Source and Preparation

The experiments were conducted during the summer season which coincided with silver perch maturation time after they were exposed to a low temperature in the winter months in Western Australia. Three pairs of broodstock aged approximately six years with an individual weight range of 1.2-3.1 kg for males and 2.5-3.7 kg for females were obtained from Curtin Aquaculture Research Laboratory, Curtin University, Bentley (31°59'38.26"S 115°53'18.09"E). The broodstock were maintained in a semi-closed water recirculating system and fed commercial feed at a rate of 2% body weight day^{-1} containing 35% crude protein.

The mature females were marked by the soft and swollen stomach and pink-red genital papilla whereas mature males were marked by the release of the milt when gentle pressure was applied to the abdomen; these were selected for this experiment. The selected mature broodstock were then moved into a 200 litre fibreglass tank and anaesthetised with A-QUIS at 100-120 ppm solutions before hormonal injections. All broodstock were injected intramuscularly with HCG hormone at 200 $IUkg^{-1}$ body weight using a disposable syringe and a 21G x 1.25 needle to initiate spawning. Each pair of induced broodstock were placed in a 2-ton fibreglass tank until spawning (Rowland, 2004a). The water was vigorously aerated and the water temperature was maintained at 23°C. The tanks were inspected periodically to observe the spawning. As the spawning was completed, the post spawning broodstock were anaesthetised and weighed before they were returned to the rearing tank. The female weight was used for relative fecundity calculations.

Egg Quality Classification

Under the stereo microscope, the oil globule was clearly visible, located in the middle of the yolk sac of newly fertilized eggs (diameter range 2.10-2.41 mm). The eggs of silver perch were classified based on the oil globule fragmentation following the category stated by Zarski *et al*. (2011b) with minor modifications. However, only three out of four categories were observed in this research. These categories were: cat-1, a clearly visible, un-fragmented single

droplet of oil globule, cat-2, several small droplets of the oil globule along with a large one, and cat-3, a highly-fragmented oil globule in the form of several droplets (Figure 1).

Reproductive Performance of the Female Broodstock (Experiment-1)

The reproductive performance of the broodstock (n = 3) was evaluated based on the fecundity, fertilisation rate, hatching rate, and egg categories. The relative fecundity was defined as the number of recently spawned eggs divided by the female body weight (Hunter *et al*., 1992; Cerdá *et al*., 1994; Coward & Bromage, 1999). Fecundity and fertilization percentage were estimated according to established procedure (Panini *et al*., 2001) with some modifications.

For fecundity determination, the eggs produced by each individual female were initially placed in a 10 litre bucket equipped with aeration from the bottom to provide gentle agitation of the eggs. A subsampling of 10 mL was taken and the number of eggs were counted before conversion to the bucket volume. The relative fecundity was then calculated and presented as a number of eggs kg^{-1} female body weight after spawning. At the same time, the fertilization rate was also estimated by counting the number of fertilised eggs divided by the total number of eggs in 10 mL subsamples.

To determine the hatching rate, eggs from the 10 litre bucket were taken in a 250-μm mesh filter and were rinsed with filtered freshwater (Aqua-pure Model-AP12S, 5 microns) and placed in a two litre beaker. A petri dish was used to scoop 100–200 eggs from the beaker which was then placed under a stereoscope. Only fertilized eggs were taken one by one with a pipette and transferred to the wells of the microtiter plates at a rate of one egg per well. The microtiter plates were then covered with a plastic lid, and placed in a controlled-temperature incubator set at 23°C until hatching. Using a stereoscope, the number of larvae were counted and the hatching rate was calculated as the number of larvae divided by the number of fertilised eggs initially loaded in the microtiter plates.

To classify the eggs, another sample of 10 mL in volume were taken from the 10 litre bucket and placed them into five petri dishes (50 mm diameter and 12 mm height). The eggs in the petri dishes were photographed under a stereo microscope using an Olympus SC30 camera with an image acquisition software, getIt, from Olympus Soft Imaging Solutions (Papanikos *et al*., 2003). The eggs were then classi-

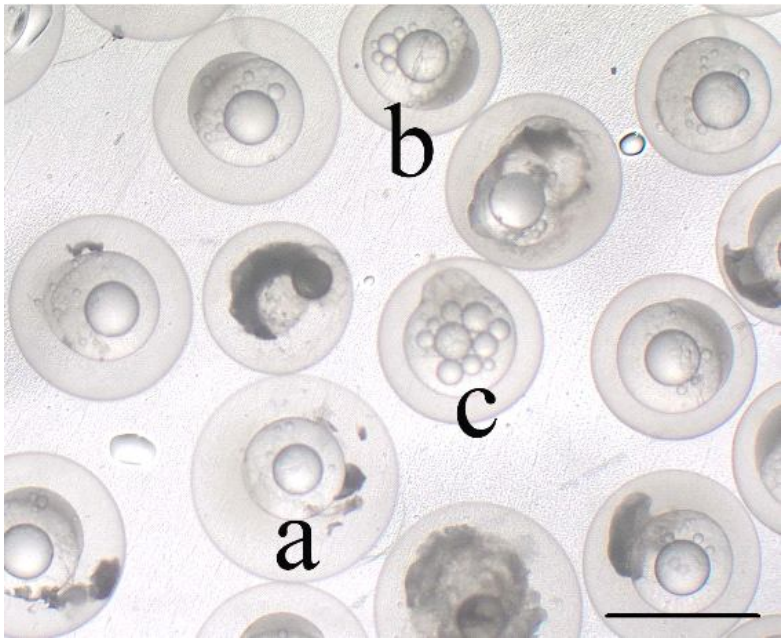

Figure 1. Different categories of ovulated eggs of silver perch with different oil globule fragmentation: category-1 (a), cat-2 (b), and cat-3 (c). Bar = 2 mm.

fied based on its oil globule fragmentation as explained earlier. From the images, the number of eggs in each egg category of each broodstock was counted and the percentage of egg category was calculated.

The Relationship Between the Degrees of Oil Globule Fragmentation and Egg Quality (Experiment-2)

Embryo and larval performance at different egg categories

The eggs from a female were sampled from the 10 litre bucket, as in fecundity determination, using a 250-μm mesh filter, rinsed with filtered freshwater, and placed in a two litre beaker (Panini *et al*., 2001). A petri dish was used to scoop 100-200 eggs from the beaker and was placed under a stereoscope and only fertilised eggs were taken one by one with a pipette and transferred into microtiter plates at a rate of one egg per well. Each egg category was placed in three separate microtiter plates as triplicates. The microtiter plates were then covered with a plastic lid, placed in a controlled-temperature incubator where the temperature was maintained at 23°C. Using a stereoscope, the survival rate of the embryo from each egg category was calculated at 10 and 20 hour post spawning (hps) and the hatching rate was calculated following the formula: the number of larvae/number of eggs in the microtiter plates ×100. The deformity rate was calculated as the number of larvae with physical deformity/the number of larvae in the microtiter plate.

Effect of egg quality on growth and survival rate

As soon as the entire batch of eggs were hatched, the larvae from each egg category (section 2.4.) were pooled and placed in five litre cylindrical glassware as stock for a growth and mortality experiments. The glassware was placed in an incubator tank where the temperature was set at 23°C as in the incubation temperature.

Two-day-old larvae were randomly taken from each holding glassware (categorised eggs) and placed in one litre beakers at 20 larvae L^{-1} stocking density in triplicates. Larvae were fed rotifers at a density of 10 ind.mL^{-1} starting at four days post hatching (dph). The density of rotifers was maintained throughout the experiment by daily adjustments whereas water temperature was set to 23°C. The faeces and debris, as well as dead fish were removed from the glass beaker every day before feeding and the mortality was recorded. At the end of the experiment at 12 dph, the survival rate was calculated while larval growth was determined by measuring the TL (mm) of ten larvae from each egg category.

Data Analysis

The IBM SPSS 24 and MS Excel 2007 for Windows software were used for the analysis of data and statistical analysis. The data was presented as mean ± S.E. The data expressed in percent was subjected to arcsine transformation before the statistical analysis was performed. The one way analysis of variance (ANOVA) followed by the Tukey's post hoc test was applied at the significant level less than 5% ($P < 0.05$) when the analysis of variance revealed statistically significant differences.

RESULTS AND DISCUSSION

Reproductive Performance of the Female Broodstock (Experiment-1)

The reproductive performance of the six year-old silver perch broodstock was found to be high as displayed by all studied parameters (Table 1). The relative fecundity was high, ranging from 120,000 to 134.200 kg^{-1} of bodyweight. Besides, the fertilisation rate and the hatching rate were also high, 94.27 ± 1.28% and 87.94 ± 1.23% respectively (Table 1). The relative fecundity per kg of female of three year-old domesticated silver perch broodstock was 139,286 ± 11,405 as reported by Rowland (2004a), is comparable to the fecundity of the six year-old broodstock (132,400 ± 7,218) used in the present study (Tabe 1). However, the fertilization and hatching rates obtained in this study were much higher than reported by Rowland (2004a) at 84.5 ± 3.7% and 76.8 ± 2.8% respectively. These different results can be due to different aspects such as age, food, and level of domestication of the broodstock population. Rowland (2004a) used a three year-old F-1 broodstock at first maturation, while in this study, six year-old F-2 broodstock was used. According to Izquierdo *et al*. (2001), the age of the fish is an aspect that affects reproductive performance, wherein the first maturation commonly results in poor performance than the older fish (Emata, 2003).

The eggs produced by these six year-old broodstock were classified as good quality eggs, since most (99%) eggs produced were classified as cat-1 and cat-2. The mean percentage of eggs in cat-1 of 78.11% was significantly higher ($P < 0.05$) than cat-2 and cat-3 at 21.26% and 0.40% respectively. Other than the egg quality, the results also confirmed that the

Table 1. Reproductive performance and egg quality composition of silver perch broodstock (n = 3) domesticated in a semi-closed recirculation system

Broodstock (female)	Fecundity (eggs kg^{-1})	Fertilisation rate (%)	Eggs quality			Hatching rate (%)
			Cat-1	Cat-2	Cat-3	
1	145,200	96.38	78.16	21.31	0.50	90.38
2	132,000	94.47	73.87	25.48	0.00	86.48
3	120,000	91.95	82.30	16.98	0.71	86.96
Average	132,400 ± 7,22	94.27 ± 1.28	78.11 ± 2.44^{c}	21.26 ± 2.45^{b}	0.40 ± 0.21^{a}	87.94 ± 1.23

Note: The eggs quality data labelled with different superscript in the same row of egg quality were statistically different ($P < 0.05$)

reproductive performance of domesticated broodstock reared in captivity for about six years was still high as indicated by the high viability of larvae. These findings confront the previous study by Thurstan & Rowland (1994) who proposed that the fecundity of silver perch broodstock may be reduced after five years reared in captivity.

As in most other cultured fish, silver perch showed segregation in oil globules and this phenomenon is may related to the level of stress occurred during oocyte maturation and spawning process. The process of oocyte maturation is known to incorporate different components such as yolk protein and oil globule (Bromage *et al*., 1992). Another study showed that the process of maturation can be disturbed by different activities in controlling reproduction such as through hormonal stimulation or the manipulation of environmental conditions (Zarski *et al*., 2011b). As a result, the segregation of oil droplets during oocyte maturation is occurred as has been reported for *Perca fluviatilis* (Zarski *et al*., 2011b), *Anguilla anguilla* (Palstra *et al*., 2005), *Lota lota* (Paliñska-Zarska *et al*., 2014), *Salmo truttafario* (Mansour *et al*., 2007), and *Oncorhynchus mykiss* (Ciereszko *et al*., 2009). The segregation of oil globules in a different category of fragmentation has been identified as affecting egg quality and regarded as an indicator of egg quality (Zarski *et al*., 2011b).

The Relationship Between the Degrees of Oil Globule Fragmentation and Egg Quality (Experiment-2)

The egg quality not only affects embryonic development and their success in hatching, but also affects larvae growth and mortality in the beginning of the developmental stages (Houde, 1974). The survival rate of embryos in all egg categories was high during the incubation period. No mortality was observed until 10 hps and only a few embryos have die at 20 hps. A significant difference in embryo survival from different egg categories was detected at 20 hps (Table 2), whereas no significant difference in the deformity rate among the egg categories was found. The survival rate of eggs in cat-1 and cat-2 showed higher viability than cat-3 eggs as indicated by their high survival rate of the embryo during incubation, especially at 20 hps. Further evidence was shown in the hatching rate where only about 60% of eggs in cat-3, while in cat-1 and cat-2 the hatching rate were higher at 93% and 85% respectively. Even if the embryo survival and hatching rate differed according to egg category, the frequency of developmental abnormalities was low in each egg category (0.0%-0.5%) and there was no difference between egg categories. However, the indication of higher deformation at higher oil globule fragmentation, as in cat-3, may indicate that these eggs were of poorer quality than those in cat-1 and cat-2. Zarski *et al*. (2011b) reported that viability of Eurasian perch eggs at cat-3 was lower compared to cat-1 and cat-2. On the other hand, Ciereszko *et al*. (2009) reported the inconsistency of the relationship between oil fragmentation and egg quality of rainbow trout where the high fragmentation of the oil globule did not always result in lower egg quality.

According to Abi-ayad *et al*. (2000), the oil content of eggs is constant during embryogenesis and is not used until the larvae's metamorphosis stage is reached. This means that oil globule fragmentation may not directly affect the survival rate of the embryo during incubation. A mechanism may act as a stressor resulting in higher mortality during the incubation phase and lower the hatching rate of eggs in cat-3. One possible explanation is the process of ovulation is too early and some eggs do not complete the normal final maturation of the oocytes as in category-1 and 2. This explanation is also reported by Zarski *et al*. (2011b) where early ovulation could

Table 2. The percentage of survivors of silver perch embryos at different periods of incubation, hatching rate, and the rate of deformity of newly hatched larvae at different categories of egg quality

Egg category	Mean survival rate (%)		Hatching rate	Deformities at hatching (%)
	10 hps	20 hps		
Cat-1	100.00 ± 0.00[a]	95.33 ± 0.00[b]	93.33 ± 0.00[b]	0.00 ± 0.00[a]
Cat-2	100.00 ± 0.00[a]	90.00 ± 0.00b[b]	85.00 ± 0.00[b]	0.33 ± 0.003[a]
Cat-3	100.00 ± 0.00[a]	72.33 ± 1.76[a]	60.33 ± 0.00[a]	0.50 ± 0.003[a]

Note: The data labelled with different superscript in the same column were statistically different ($P<0.05$). hps = hour post spawning

have disturbed the final maturation of the oocyte in Eurasian perch which resulted in a low quality of eggs in cat-3 and cat-4 as indicated by the low survival rate during incubation, low hatching rate, low growth rate, and high mortality of the new larvae.

The visibility of oil globule fragmentation during larval development as noted at 8 dph (Figure2), may suggest that the fraction is permanent until all oil is absorbed completely. This is different from Eurasian perch where the fraction of the oil globule may be merged, partly or totally, immediately after eggs were ovulated and get in contact with water at spawning (Zarski *et al*. 2011b) and in medaka, *Oryzias latipes* (Iwamatsu *et al*., 2008). The oil fragmentation may contribute to the growth and survival rate differences of silver perch larvae from different egg categories. Related to mortality, Li & Mathias (1982) pointed out that the high mortality of Walleye larvae, *Stizostedion vitreum*, occurred during yolk sac consumption and complete absorption of oil globules.

The egg quality also affected the TL of the larvae as detected at 12 dph. The growth of silver perch larvae was clearly affected by the egg's categories. The TL of larvae in cat-1 and cat-2 (8.4 ± 0.2 and 8.3 ± 0.2 mm respectively) did not differ significantly ($P>0.05$), but they were much higher than category-3 (7.0 ± 0.1 mm) at 12 dph (Figure 3). The more fragmented the oil globule, the lower the TL achieved.

Figure 2. The appearance of oil globule fragmentation of silver perch larvae originating from category I (a), category II (b), and category III (c). Bar A= 500 μm, B= 500 μm, and C= 200 μm.

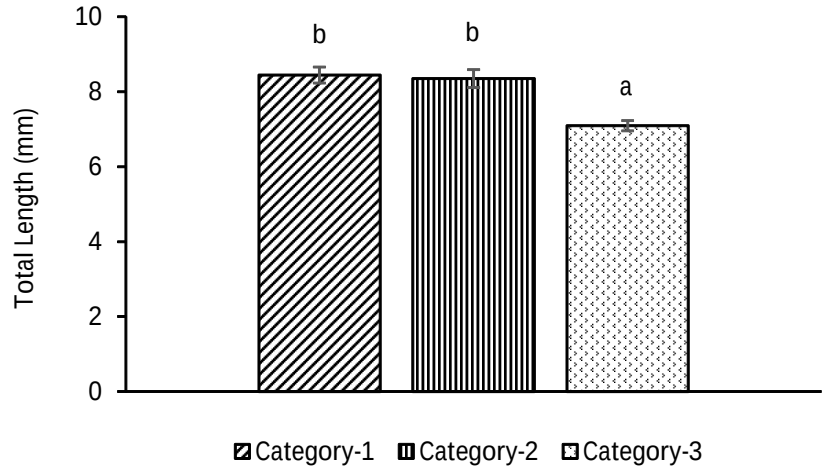

Figure 3. The total length (TL) of silver perch, *Bidyanus bidyanus* larvae resulting from different egg quality at 12 days post hatching (dph). The same letter over the bars indicates no significant difference ($P > 0.05$).

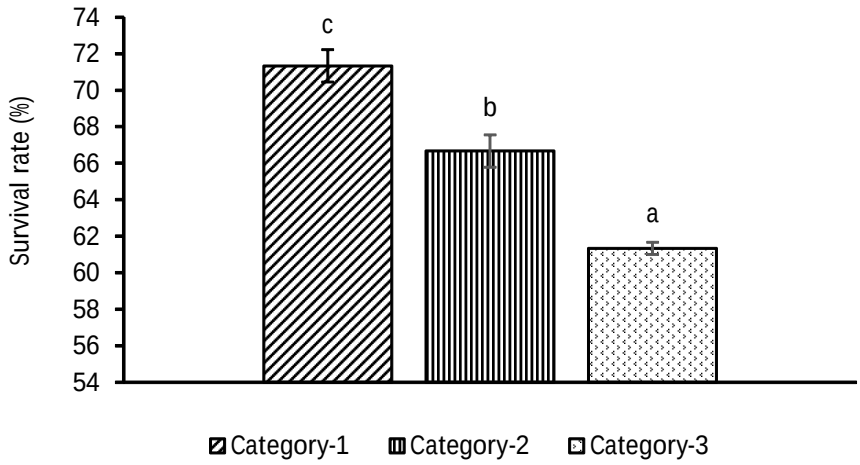

Figure 4. Survival rate (%) of silver perch, *Bidyanus bidyanus* larvae from different egg categories at 12 days post hatching (dph). Different labels over bars designates significant difference ($P < 0.05$).

The same was true with the survival rate. After 12 days of the rearing period, the lowest survival rate of 61.3 ± 0.3% was noted in egg cat-3, which was significantly ($P < 0.05$) lower than the survival of cat-1 and cat-2 at 71.3 ± 0.9% and 66.7 ± 0.9%, respectively (Figure 4). The survival rate of cat-1 did not show any significant difference ($P > 0.05$) when compared to cat-2. It has been reported that the oil globule contained vitamin A and nutrient ingredients of high caloric value (Iwamatsu *et al*., 2008), which is crucial for larvae development during the transition from endogenous to exogenous feeding (Iwamatsu *et al*., 2008). The process of assimilation of fragmented oil globules into the larval body may be obstructed and reflected in small TL and low survival rates at 12 dph. This has also been the reason for low viability and high growth variability in Eurasian perch (Zarski *et al*., 2011b).

CONCLUSION

The results suggest that domesticated (F-2) silver perch broodstock reared in captivity for about six years are still viable with acceptable reproductive performance and high quality of eggs. An oil globule fragmentation test is recommended as a valuable tool for evaluating the egg quality in silver perch.

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