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Coral platy fish *Xiphophorus maculatus* hormonal induction to improve mass spawning efficiency

Induksi hormon untuk meningkatkan efisiensi pemijahan massal ikan plati koral *Xiphophorus maculatus*

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ABSTRACT

Coral platy fish has a unique reproduction due to ovoviviparous (live-bearer) reproduction. The large-scale production of coral platy fish has several constraints due to the inconsistent seed birth period, which causes variations in the fry size. This makes it difficult for ornamental fish cultivators for production process efficiency and market criteria fulfillment that demands size uniformity. This study aimed to synchronize the broodstock birth period of coral platy fish by testing the hormone oxytocin and prostaglandin-2 α (PGF2α) through immersion methods with different durations. This study used a factorial randomized design with 21 treatments and 3 replications for each hormone type. The PGF2α hormone doses used were 0; 0.01; 0.1 and 1 mL/L, while the oxytocin hormone doses used were 0; 0.1; 0.2; 0.4 mL/L with immersion duration of 4, 8, and 12 hours, respectively. The results showed that the treatment dose of 1 mL/L PGF2α for 12 hour immersion had a significant effect (P<0.05) compared to other treatments, the immersion group with 12 hour duration obtained a significant difference to the other treatments, both at the percentage of broodstock giving birth and the number of seeds. The hormone treatment had no significant effect on broodstock and seed survival (P>0.05).

Keywords: mass induction, oxytocin, prostaglandin-e2 α (PGF2α), mass birth, livebearer

ABSTRAK

Ikan plati koral memiliki reproduksi yang unik karena bereproduksi secara ovovivipar (*live-bearer*). Produksi ikan plati koral dalam skala besar dihadapkan kendala akibat waktu kelahiran anak yang tidak serentak yang menyebabkan keberagaman ukuran anak ikan plati koral. Hal ini menyulitkan para pembudidaya ikan hias untuk efisiensi proses produksi dan memenuhi kriteria pasar yang menuntut keseragaman ukuran. Penelitian ini bertujuan untuk menyeragamkan waktu kelahiran anak induk ikan plati koral dengan uji coba pemberian hormon oksitosin dan prostaglandin-2α (PGF2α) melalui metode perendaman dengan durasi waktu yang berbeda. Penelitian ini menggunakan rancangan acak faktorial dengan 21 perlakuan dengan 3 kali ulangan untuk masing-masing jenis hormon. Dosis hormon PGF2α yang diuji adalah 0; 0,01; 0,1 dan 1 mL/L, sedangkan dosis hormon oksitosin yang digunakan adalah 0; 0.1; 0.2; 0.4 mL/L dengan masing-masing lama perendaman 4, 8, dan 12 jam. Hasil yang diperoleh menunjukkan bahwa perlakuan dosis 1 mL/L PGF2α dengan lama waktu perendaman 12 jam memberikan pengaruh yang nyata (P<0.05) dibandingkan dengan perlakuan lainnya. Kelompok perendaman dengan durasi 12 jam memberikan perbedaan yang nyata terhadap lama perlakuan lain, baik pada parameter persetase induk melahirkan maupun jumlah anak yang dilahirkan. Perlakuan hormon tidak memberikan pengaruh yang nyata terhadap kelangsungan hidup induk dan anak yang dilahirkan (P>0.05).

Kata kunci: induksi massal, Oksitosin, prostaglandin-2 α (PGF2α), kelahiran masal, *livebearer*

INTRODUCTION

Platy coral fish *Xiphophorus maculatus* is one of the ornamental fish from Pociliidae family, which has small size and flat shape with lengthened caudal fin in male fish as a sexual dimorphism character (Gómez-Gonzales *et al.,* 2014). Platy fish majorly has bright colors, such as red and orange (Froese & Pauly, 2007; Bano & Serajuddin, 2017). This fish originates from Mexico (Dávila-Camacho *et al.,* 2018) and introduced to around 18 countries, namely, Indonesia, Singapore, Japan, Nigeria, USA, Brazil, and Australia (Magalhães & Jacobi, 2017), besides capable of adapting to a worse environment (Maddern *et al*., 2011). This fish is utilized as a larvivorous fish to handle mosquito larvae in tropical and sub-tropical countries (Surendranath *et al*., 2018). The market price in Indonesia ranges Rp1,000–1,500 per fish. KKP (2015) stated that the production of platy fish in Indonesia in 2014 reached 44.2 million fish and was predicted to continuously increase.

The coral platy fish include in a live-bearer fish or giving birth fish group (Froese & Pauly, 2007; Tolon, 2018). This fish can produce seed many times during mating season; the period between pregnancy/birth is 25-35 days, averagely around 28 days (Shahjahan *et al*., 2013; Yang *et al*., 2012). Most live-bearing fish have asynchronous embryo development and birth process, therefore the embryos contained have various sizes (Norazmi-Lokman *et al*., 2016). This condition causes the inhibition of mass breeding process due to various seed sizes produced.

The embryo develops in the ovarian follicle during the developmental phase and is born as a seed (Bone & Moore, 2007) with the next fertilization process is thought to occur after the previous birth process (Yang *et al*., 2012). This condition causes the seed age is varied, which potentially complicates the rearing process in terms of live feed availability, seed sustainability, mainly for the experimental requirement in the laboratories (Norazmi-Lokman *et al*., 2016), difficult planting pattern implementation, and unfulfilled coral platy fish demand in the international market. This condition causes inefficient production, especially in a large scale, thus requiring an effort to unify the birth period at one period.

Hormonal influence is closely related to brain cell mechanisms, mainly on the reproductive behaviour in teleost and elasmobranchii group (Forlano & Bass, 2011). Therefore, a certain utilization of hormones potentially becomes an effort for artificial birth. Several potential hormones utilized are prostaglandin (PGF2α) and oxytocin. The PGF2α hormone acts in improving the uterus layer contraction to rapidly make the uterus contract and stimulating the nucleus in germinal vesicle that migrates into the corner part until ovulation process (Jamlaay *et al*., 2013; Sugimoto *et al*. 2015; Baek & Lee, 2019). In the induction experiment of PGF2α on snakehead broodstock fish with different doses obtained a shorter ovulation period and increased number of eggs with the dose of 0.9 mL/kg broodstock.

In mammals, oxytocin hormone stimulates strong contraction in the uterus wall, thereby facilitating the birth process (Arrowsmith & Wray,2014; Knobloch & Grinevich, 2014). In fish, oxytocin hormone is reported to be utilized as an *Ovaprim*® for artificial spawning process in sangkuriang catfish. The experiment of hormone administration with 75% oxytocin and 25% *Ovaprim*® through broodstock injection can produce the shortest ovulation period, namely, 9 hours and 23 minutes (Mayyanti, 2013). Based on some studies above, this study was performed as an effort to unify the seed birth period of coral platy fish with different hormone inductions, namely, oxytocin and prostaglandin-2α (PGF2α), through immersion bath method with different durations.

MATERIALS AND METHODS

Experimental design

The experimental design used is a factorial randomized design with two test levels, namely, different hormone types and doses with different immersion duration periods against the uniformity of broodstock birth period observed for 7 days after hormonal immersion. The hormone types used were oxytocin with the doses of 0.1 mL/L, 0.2 mL/L, and 0.4 ml/L and prostaglandin (PGF2α) with the doses of 0.01 mL/L, 0.1 mL/L, and 1 mL/L, and control by adding non-hormonal treatment; each dose was tested at the immersion durations of 4, 8, and 12 hours. This study contained 21 treatments with three replications which performed serially (time-series) based on the replication of each hormone treatment. The number of broodstocks used on each replication was 10 broodstocks, thus the total number of broodstocks used was 12 broodstocks for each hormone type with different period. The hormones

used had a hormonal injection specification for animal with the commercial brand of *Oxytocin-10* (10 IU) and *Capriglandin* (5.5 mg dinoprost tromethamine, 12.0 mg benzyl alcohol).

Experimental object

This study used pregnant female broodstocks of coral platy fish that were ready to give birth. Pregnant broodstocks with the average weight of 2.08±0.02 g and average length of 4.03 ± 0.02 cm were selected. These broodstocks were obtained from the ornamental fish cultivator in Parung, Bogor, West Java.

Container preparation

The container used in this study was 60×40×30 cm³ aquarium as much as 12 aquaria for broodstock rearing, and 40×30×25 cm³ aquarium as much as 12 aquaria for larval rearing container. The aquarium disinfection was performed using 40 mg/L potassium hypochlorite and neutralized using 20 mg/L sodium thiosulfate for four hours. The aquarium was then rinsed with clear water and dried for 24 hours. Aquarium was filled with water until 25 cm height equipped with aeration. As an effort to minimize broodstock fed the seeds, a breeding trap modification was installed at the end part of aquarium.

The container used during immersion was 15×15×25 cm³ aquarium. After the container was ready, 1 L water was filled, then hormones were taken by syringe and distributed into the immersion aquarium based on each treatment dose. The aquarium was aerated and stood for 10 minutes. Fish were moved into the aquarium and stood based on the immersion duration treatment.

Broodstock rearing

Fish were reared in a rearing container by maintaining the optimum environmental condition. Water exchange was performed once in 2 days. Water quality measurement containing temperature, pH, and DO was performed every day, whole ammonia, nitrite, and nitrate were measured once in 3 days regularly and scheduled. Fish were fed 3 times a day with *Tubificidae* in *ad libitum* method (Velasco-Santamaria & Corredor-Santamaria, 2011; ŞAHİN *et al*., 2017). Fish were observed their behaviours and number of seeds produced. Moreover, the broodstock survival rate was calculated at the end of rearing.

Seed harvesting

Seeds were harvested when each broodstock gave birth with 48 hour observational period. Fish seeds were carefully harvested by syphonization, then performed a total larvae calculation, before moving into a separated container to identify the larval survival rate after 10 days of rearing.

Parameters

The parameters observed in this study comprised percentage of broodstock giving birth, number of seeds, seed and broodstock survival rate, and water quality during rearing.

Percentage of broodstock giving birth

Total broodstock birth was noted every 4 hours during the observation period and accumulated at the end of the observation period. The number of seeds were noted and differed based on each broodstock.

The number of seeds

The number of seeds was calculated every 4 hours during the observation period. The number of seeds were noted and differed based on each broodstock.

Survival rate (SR)

The survival rate is a ratio of total living broodstock/seed at the final rearing and total fish/seed at the initial rearing.

Note :

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

SR = Survival rate (%)
N0 = Total fish stocked during initial rearing (fish)
Nt = Total fish living during final rearing (fish)

Data analysis

The data obtained were tabulated and analyzed. Data obtained were tabulated using MS. Excel and analyzed using analysis of variance at 95% confidence level, then continued with Tukey test using SPSS 25.0, when there is any significant different response among treatment.

RESULTS AND DISCUSSION

Results

Percentage of broodstock giving birth (PIM)

The percentage of broodstock giving birth (Figure 1) was obtained from the percentage of total final broodstock number that gave birth during the observation period. Based on the experiment

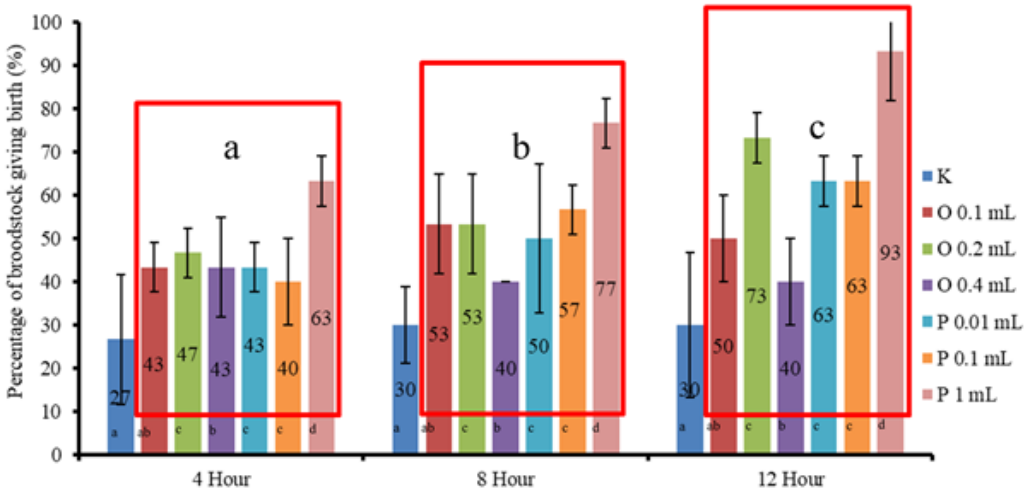

Figure 1. The percentage of coral platy fish broodstock giving birth immersed with prostaglandin and oxytocin hormones with different doses and immersion durations. Note: K (control), 0.1 mL/L oxytocin, 0.2 mL/L oxytocin, 0.4 mL/L oxytocin; 0.01 mL/L prostaglandin; 0.1 mL/L prostaglandin; and 1 mL/L prostaglandin, with the respective immersion durations of 4, 8, 12 hours. Different letters on the bar show a significant difference (P<0.05). Values presented are the average value.

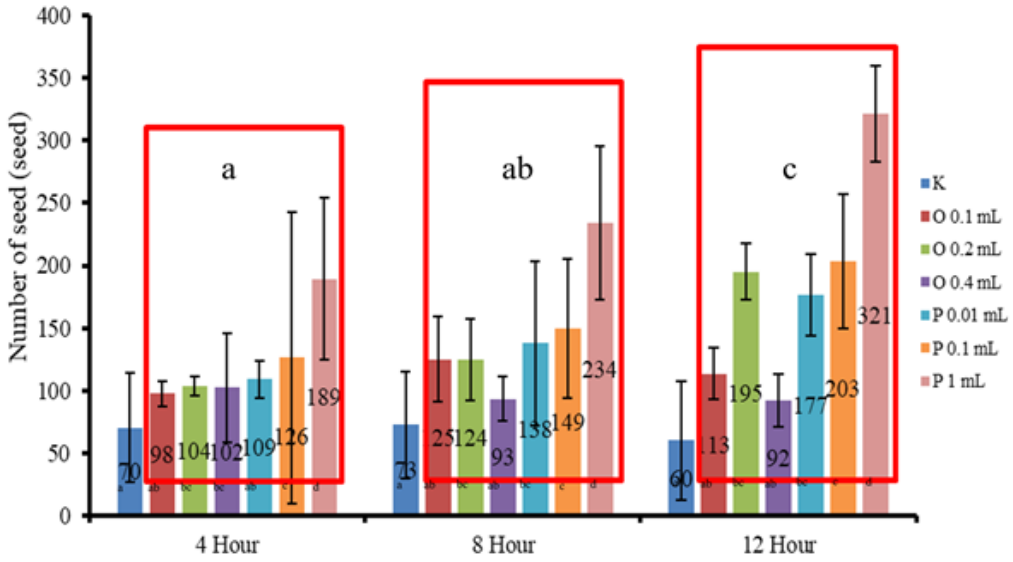

Figure 2. The number of coral platy fish *X. maculatus* seeds in 7 days after immersion. Note: K (control), 0.1mL/L oxytocin, 0.2 mL/L oxytocin, 0.4 mL/L oxytocin; 0.01 mL/L prostaglandin; 0.1 mL/L prostaglandin; and 1 mL/L prostaglandin, with the respective immersion durations of 4, 8, 12 hours. Different letters on the bar show a significant difference (P<0.05). Values presented are the average value.

results, each treatment either doses or immersion duration had a significant difference (P<0.05) against the percentage total of broodstock giving birth. However, there was no interaction between hormones and immersion duration treatments.

The number of seed

The number of seeds produced by broodstock in each treatment is presented on Figure 2. This figure shows each treatment of hormone dose and immersion duration which was significantly different (P<0.05) against the number of seeds. However, there was no interaction between hormone and immersion duration factors obtained.

The survival rate of coral platy fish broodstock (SRi) and seeds (SRa)

The survival rates of coral platy fish broodstock and seeds (Table 1) were obtained from the ratio percentage of total final broodstock/seeds against total initial broodstock/seeds, as each of which

Table 1. The survival rate of (SRi) coral platy fish *X. maculatus* in 7 days after giving birth

Broodstock survival rate (%)	K	O 0.1 mL	O 0.2 mL	O 0.4 mL	P 0.01 mL	P 0.1 mL	P 1 mL
4 hours	100	100	100 ± 7	100	100	100	97 ± 6
8 hours	100	100	100 ± 7	100	100	97 ± 6	100
12 hours	100	100	100 ± 7	100	100	100	100
Seed survival rate (%)	K	O 0.1 mL	O 0.2 mL	O 0.4 mL	P 0.01 mL	P 0.1 mL	P 1 mL
4 hours	80.00	82.00	77.30	88.00	83.48	84.94	82.53
8 hours	88.30	79.70	83.00	83.30	74.63	85.90	82.90
12 hours	86.00	83.00	84.30	84.70	87.57	87.68	88.47

Note: K (control), 0.1mL/L oxytocin, 0.2 mL/L oxytocin, 0.4 mL/L oxytocin; 0.01 mL/L prostaglandin; 0.1 mL/L prostaglandin; and 1 mL/L prostaglandin, with the respective immersion durations of 4, 8, 12 hours. There are no treatments that showed a significant difference against parameter tested (P>0.05). Values presented are in the average and standard deviation.

Table 2. Water quality in coral platy *X. maculatus* broodstock rearing

Water quality parameter	Treatment				References
	PA	PB	PC	PD	
pH	7.3–7.5	7.4–7.6	7.4–7.7	7.3–7.6	6.5–9 (Boyd, 1990)
Temperature (˚C)	26–27	26–27	26–27	26–27	26˚C (Yang *et al.,* 2012)
DO (mg/L)	3.6–5.7	3.8–6.3	3.5–6.2	3.4–4.3	>3 (Boyd, 1990)
Ammonia (mg/L)	0.009	0.03	0.03	0.03	<3 (Yang, 2012)

Table 3. Water quality in coral platy *X. maculatus* seed rearing

Water quality patameter	Treatment				References
	PA	PB	PC	PD	
pH	7.4–7.6	7.4–7.8	7.3–7.6	7.5–7.6	6.5–9 (Boyd, 1990)
Temperature ℃)	26–27	26–27	25–27	26–27	25–27 (Boyd, 1990)
DO (mg/L)	2.7–3.5	2.3–4.1	3.2–4.7	3.2–4.1	>3 (Boyd, 1990)
Ammonia (mg/L)	0.009	0.009	0.009	0.009	<1 (Zakaria, 2003)

was observed in 7 days after immersion and 10 days after giving birth. The fish survival rate showed no significant difference (P>0.05) on all treatments.

Water quality parameter

Water quality data were used as supporting data for environmental condition against broodstock and larvae of coral platy fish during study. The result data of water quality measurement is presented on Table 2. The results of water quality observation in broodstock rearing container showed the same value on all PA treatments which are lower than other treatments. The water quality value was also in the optimum range that supported the fish life (Boyd, 1990).

The water quality observation results in seed rearing container (Table 3) during 10 days of observation showed that pH, temperature, and ammonia was similar on all treatments that supported the fish life (Boyd, 1990). Meanwhile, the lowest range on the oxygen content for PA and PB treatment were smaller that other treatments based on the reference standard (Boyd, 1990).

Discussion

The hormone used in this study aimed to stimulate the coral platy fish broodstock to give birth. The experimental results between hormones at various doses showed a significant difference in the percentage of broodstock giving birth (Figure 1). In the administration of the oxytocin hormone, the treatment dose of 0.02 mL/L showed a significant difference among other doses (increased by 100% compared to control), thereby can be stated that hormones play a role in

the uterine membrane contraction and coral platy fish broodstock stimulation to give birth. This was based on Muchlisin *et al*. (2014) and Khajehei (2017), who stated that oxytocin is very effective in contracting arterial and venous blood vessels, making the oxytocin concentration in the blood increases greatly during giving birth. In mammals, this hormone mediates the increased uterine myometrial contractility (Maiti *et al*., 2011; Tica *et al*., 2011). Increased oxytocin hormone dose has a positive impact on the broodstock physiological condition to a certain point, then decreases or becomes negative. This was identified from the administration of 0.2 mL/L oxytocin hormone; the percentage of broodstock giving birth decreased on all immersion durations. Several negative effects on the seeds were reported due to hormone hyperstimulation (doses/exposure period) as the increased broodstock contraction frequency (Xu *et al*., 2017), therefore, suspected to provide a pressure for coral platy fish broodstock, then failed to give birth.

In fish, oxytocin hormone is often used for *Ovaprim*® and *spawnprime*® mixture as a formula to accelerate the broodstock ovulation process. However, the use of 100% oxytocin in ovulation process does not provide effective results (Islami *et al*., 2017). This is contradictory to oxytocin hormone as the most widely used ovulation booster (Magon & Kalra, 2011). This is because most types of fish do not have a uterus. Only fish that have an ovoviviparous reproduction type, such as coral platy fish have a uterus. The oxytocin hormone used in this study was a synthetic oxytocin hormone. The oxytocin hormone works by stimulating the uterus contractions during the birth process. Oxytocin is synthesized by the paraventricular nucleus nerve cell body which causes the smooth muscle of the uterus contract in the final phase of pregnancy (Priyadarshi *et al*., 2020). This makes the coral platy fish broodstock can spawn simultaneously in 48 hours after immersion.

The PGF2α hormone treatment with a dose of 1 mL showed the best results and was significantly different from other doses (increased by 269% compared to control). This means that the PGF2α hormone plays a role in the uterus layer contraction and coral platy fish broodstock stimulation to give birth. This was based on Moallem *et al*. (2013), who stated that the increased concentration of PGF2α hormone in blood will induce the uterus layer contraction, thereby accelerating the birth process. The experimental results of all hormones with various doses indicated 1 mL treatment dose of PGF2α hormone was the best treatment and was significantly different from other treatments. Based on this result, the administration of oxytocin and PGF2α hormones can increase the birth process stimulation in coral platy fish broodstock, although the best performance was obtained from the administration of PGF2α hormone as increased by 169% compared to control. The PGF2α hormone is often used in animals based on the principle of the hormone administration which can lyse or degrade the corpus luteum followed by decreased progesterone secretion that causes reproductive cycle alteration (Kim *et al*., 2015; Plewes *et al*., 2020).

Along with the increased number of broodstock giving birth due to the treatment given, hormone administration also resulted in an increased number of seeds produced with a similar pattern. The administration of oxytocin hormone at 0.02 mL/L dose showed a significant difference to other doses (increased by 108% compared to control) and indicated a decreased performance at higher doses. The similar condition occurred in the administration of PGF2α hormone; 1 mL dose treatment had the best result and a significant difference against other doses (increased by 267% compared to control). Immersion duration had a significant difference against the increased number of seeds produced by coral platy fish broodstock. The 12-hour immersion treatment could increase the number of seeds by 40% compared to control treatment; thereby a longer hormone exposure to broodstock increases the broodstock birth stimulation (Figure 2). There was no interaction between hormone dose treatments and immersion duration associated with increased number of seeds produced by coral platy fish broodstock.

This experiment also proves that the immersion duration has a significant effect in the birth process stimulation improvement on coral platy fish broodstock. The 12 hours treatment showed a significant difference among other treatments (increased by 33% compared to control). The longer hormone exposure period, the more broodstock giving birth stimulated (Figure 1). This indicates that a longer the immersion duration to broodstock, the higher the amount of hormone absorbed by the body, resulting in the increased number of broodstock giving birth and seeds produced. However, no interaction was found between hormone dose treatment and

immersion duration associated with the increased percentage of broodstock giving birth. Until now, there are no literatures related to the immersion duration of hormones and number of broodstock giving birth along with its mechanism. There are also no literatures stated about the relationship between the immersion duration in oxytocin and PGF2α hormones. The hormones presented in the immersion medium were thought to be absorbed by the coral platy fish broodstock through gills and skin, then entered the bloodstream and circulated to the target organs. The action mechanism of the solution by the immersion method is commonly through diffusion into skin, gills and digestive organs (Pittman *et al*., 2013; Rosmaidar *et al*., 2014). This mechanism is similar to the mechanism of recombinant growth hormonal induction in carp through immersion (Ratnawati, 2012). The absorption of dissolved components in water through the gills is usually quite large. The absorption through the digestive tract is only small, although the dissolved components in water that enter through the digestive tract are quite large, while those entering through skin are relatively small. Thus, hormone doses and immersion duration greatly affect the successive hormonal action, although there was no interaction between both factors in this study.

The physiological alteration in the broodstock immersed with hormones was uterus wall contraction followed by the birth process. This could occur after PGF2α and oxytocin hormones reached the required concentrations in blood as the doses used is commonly associated with the treatment duration. High doses are commonly applied in a short period, while low doses are applied in a long period (Zairin *et al*., 2002). This is thought to be a positive correlation factor caused by dose and immersion duration in the percentage of broodstock giving birth (Figure 1). Norambuena *et al*. (2012) stated that the higher PGF2α hormone dose, the faster ovulation time achieved. Factors influencing the effectiveness of hormones in the body include hormone dose, hormone application, feed quality, feeding time, stress, species, and fish size (Weatherlay & Gill, 1987; Phaseari, 2013; Sinjai *et al*., 2014).

The hormonal induction did not have a negative effect on the broodstock or larvae survival rate of each treatment, as observed from the survival rate of broodstock in 7 days after immersion was 90–100% (Figure 2) and larvae was 74.63–90.29% (Table 4). On day 10, similar condition was found in Davoodi *et al*. (2019). The statistical test results showed that each treatment was insignificantly different ($P<0.05$). The percentage value indicated that the hormone dose was not toxic for broodstock and did not affect the broodstock behavior during observation. This condition also applied to the survival rate of seeds (SRa) which showed that the hormone induction to increase the ovulation process had no effect on the survival rate and behavior of seeds during observation. This means that hormone treatment only influences the broodstock to increase ovulation and does not have an impact on the seeds produced. The water quality in this study represented by temperature, DO, pH, and ammonia was in a normal range and tolerable by coral platy broodstock fish (Albornoz-Garzón & Villa-Navarro, 2017) and seeds to support the broodstock and seed lives.

CONCLUSION

The results obtained from this study showed that PGF2α hormone could improve the birth period uniformity with the best dose of 1 mL/L PGF2α and 12 hour immersion duration, while the oxytocin hormone administration could improve the birth period with the best dose of 0.2 mL/L and 12 hour immersion duration (C3). From both hormones, PGF2α hormone is more effective than oxytocin hormone.

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