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The Influence of Fertilization Media pH towards P.hypophthalmus Eggs (*Pangasionodon hypophthalmus*) Fertilization

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Abstract : The purpose of the study was to find out the most suitable pH for P.hypophthalmus eggs (*Pangasionodon hypophthalmus*) fertilization. The setting of the study was the Freshwater Aquaculture Center or *Balai Perikanan Budidaya Air Tawar* of which location was Jambi, Sumatera in August 2016. The weight of the male fish was between 1.45 – 1.91 kilograms while that of the female fish was 4.5 kilograms. The study used complete random design with four different pH treatments namely pH 7 (control), pH 6, pH 8, pH9 and pH10; each of the pH treatments were repeated three times. The parameters being observed were degree of fertilization and hatching. The supported parameters were sperm density, motility, oocyte diameter and Testicle/Gonadal Somatic Index. The findings of the study were the highest percentage of fertilization was during pH8 treatment or 98.99±1.75 and the lowest percentage of fertilization was during pH 6 treatment or 81.21±9.23. In addition, the highest percentage of hatching was during pH 8 treatment or 47.75±15.13 and the lowest percentage of hatching was during pH 10 treatment or 29.14±8.50. The average sperm density was $3.07x10^9$ and the sperm motility was >86.66%. oocyte diameter in final gonad maturation stadium was 0.80-1.00 millimeter and 84.84%. Finally, the average Testicle/Gonadal Somatic Index (GSI) was 4.72.
Keywords : Pangasionodon hypophthalmus, pH, Fertility.

Introduction

P.hypophthalmus cultivation has been growing rapidly in Indonesia, more specifically in Jambi and Riau. The need for *P.hypophthalmus* seed is increasing rapidly. Besides being able to grow fast, these fish are able to adapt to extreme water condition such as lack of oxygen and low pH[1]. *P.hypophthalmus* cultivation may be in the form of floating net on the river, a pond, or even on marginal land such as peatlands and marshes where the water has low pH levels. P.hypophthalmus hatchery use water of which source is water catchment or wetlands that have low pH.

Based on the facts, it is important to conduct a study that analyzes the influence of fertilization media pH towards P.hypophthalmus eggs (*P.hypophthalmus*) fertilization. Therefore, Therefore, the researchers are interested in conducting a study that describes the influence of fertilization media pH towards fertilization of P.hypophthalmus eggs (*P.hypophthalmus*).

Methodology

The study was conducted between August and October 2016 di in Fish Hatchery and Health Laboratory and the environment of the Freshwater Aquaculture Center in Jambi.

P.hypophthalmus

The type of fish used in the study was adult P.hypophthalmus from the Freshwater Aquaculture Center of Sungai Gelam; there were 3 adult male fish and 5 adult female fish as the samples. The fish had been selected previously and had the same lineage. The P.hypophthalmus used was adult fish and had mature gonads. The weight of the female fish was > 2.5 kilograms and > 2 years old, while the male fish was > 1.5 kilograms and > 1.5 years old.

Successful fertilization is heavily influenced by the quality of both male and female adult fish. Quality of sperm and oocytes were the indicators of the fish quality. The parameters of sperm quality were concentration, motility, viability and Gonadal/Testicle Somatik Index (GSI). On the other hand the parameters of oocyte quality were oocyte diameter, egg nucleus and adhesion.

Fertilization Media pH

The fertilization media were solution made from the combination between citric acid ($C_6H_8O_7$) and aquades to make solution with acidic pH and the combination between CaOH and aquades to make solution with alkaline pH. The pH of the solutions was pH 6,7,8,9 and 10. All pH solutions were stirred until they were homogenous using magnetic stirer and then measured using pH meter (Merk Mettler Toledo MPC227).

Artificial Spawning

Adult, female P.hypophthalmus was selected to find ones with mature gonads. In order to take samples of the fish eggs, canulator was inserted into the fish genital so that the level of oocyte maturity could be observed; the number of oocytes being observed was 30 oocytes. The following procedures were to analyze diameter of the oocytes, their uniformity and the position of the egg nucleus/ Germinal vesicle Stadia using micromilimeter and microscope with 400 x magnifications (Olympus CX-41). The analysis was conducted by mixing the oocytes and serra solution. Serra solution is the mixture of 99.5% Alcohol, 37% Formaline and Acetic Acid / Glacial Acetic Acid) with ratio of 60: 30: 10. On the other hand, the adult, male fish ready for spawning would produce thick, white semen when one massages its abdomen to its genital opening. Sperm concentration was analyzed using Hemacytometer (the brand was Thoma from Japan) and motility was analyzed using a microscope with 1000x magnification. The adult, female fish being selected was injected with hormone called Gonadotrophin Realising Hormone Analog / GnRH (the brand was ovaprim manufactured by Syndel Laboratories LTD) with total dose of 0.5 milliliter/ kilogram of fish. The hormone was injected twice on the fish back/ dorsal. Ovaprim has been widely used to stimulate ovulation and spawning of freshwater fish. The first dose of the injection was 1/3 of the total dose, and that of the second injection was 2/3 of the total dose. Having been injected, the eggs would ovulate six hours later.

Harvesting Egg and Sperm

Eggs and sperm were harvested by massaging the fish abdomen to its genital opening slowly. The eggs were stored in a plastic container whereas the sperms were harvested using 5 milliliter syringe (without any needle); the syringe was used to suck the sperms out of the genital opening slowly. Both the equipment and containers should be clean and dry to prevent the eggs and sperms from being active due to water contamination. The sperms were diluted using 0.9% NaCl where the ratio was 1:4 to preserve quality of the sperms because they were stored in refrigerator/ cool box of which temperature was 4-5^{0}C for 24 hours[2]. The purpose of diluting the sperm in NaCl/ physiological solution was to prevent the sperms from being active due to the mixture between the sperms and urine[3]. During the process, both eggs and sperms should not be exposed to direct sunlight.

Fertilization

The eggs and the sperms were stirred together slowly using chicken feathers. 30 to 40 eggs that have been mixed with the sperms were stored in their respective plastic containers of which volume was 200

milliliters. 150 milliliters of water with different pH was poured into each of the containers. It was the time when the degree of fertilization and hatching were analyzed. Fertilization Rate (FR) was the comparison between number of eggs that could be fertilized and the eggs being tested multiplied by 100%. On the other hand, hatching rate was the comparison between number of eggs that hatched and the eggs being tested multiplied by 100%

Findings and Conclusion

Quality of Fish

The quality of the adult, male fish being tested was as follows:

Table 1. Quality of the Adult, Male P.hypophthalmus

No.	Parameter	Number of Adult, Male Fish			Average
		1	2	3	
1	**Total Weight (gram)**	1450.00	1525.00	1911.50	1628.83
2	**Testicle Weight (gra,)**	54.30	113.60	56.90	74.93
3	**Testicle Somatic Index (%)**	3.74	7.45	2.98	4.72
4	**Total Length (centimeter)**	58	59	60	59
5	**Sperm Density (cell/ml)**	$3.17x10^9$	$3.34x10^9$	$2.69x10^9$	$3.07\ x10^9$
6	**Motility (%)**	>90%	>90%	>80	>86.66

The average weight of the adult, male P.hypophthalmus being tested was 1.62 kilograms. The maximum production of sperm was 4.36 milliliters/kilogram of fish[4].

The average motility was >86.66%. The sperm motility described the ability of sperm to fertilize eggs; the higher the motility, the higher the percentage of the sperm viability[5]. Sperm motility is an essential factor to determine quality of sperm and its fertilization ability[6,7,8].

A	B	C

Picture 1. Comparison between size of testicle and size of adult, male P.hypophthalmus (P.hypophthalmus)

The average sperm density was 3.07 x 109 cells/milliliter; the sperm density of each species of fish is different.

The heaviest testicle weight of the fish was 113.60 grams and the lightest was 56.90 grams. Measuring testicle weight aimed at measuring theTesticle/Gonad Somatic Index (TSI) that referred to the ratio between the testicle weight and the fish weight. The highest TSI was 7.45% and the lowest was 2.98%.

Table 2. Quality of the Adult, Female P.hypophthalmus

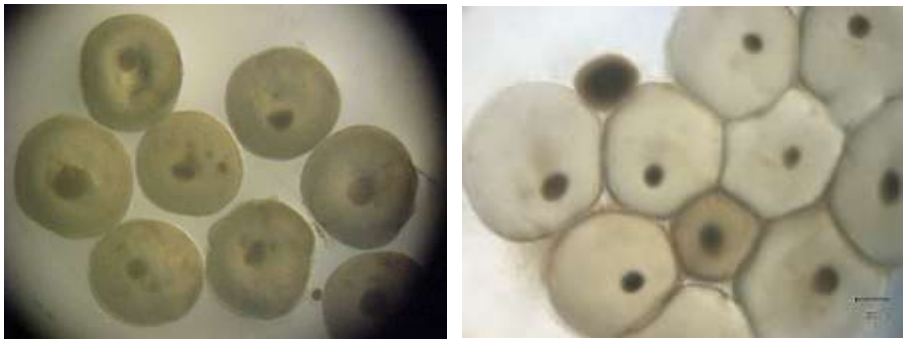
No		Parameter		Number of Adult, Female Fish				
				1	2	3	4	5
1		Total Weight (gr)		4430	5270	4500	4810	4120
2		final gonad maturation stadium (%)		66.67	56.67	84.85	45.71	67.74

Based on Table 2, the heaviest adult, female P.hypophthalmus was fish number 3 of which weight was 4,500 grams while the lightest adult, female P.hypophthalmus was fish number 5 of which weight was 4.120 grams. To determine the quality of a good adult, female P.hypophthalmus / mature gonads ready for spawning, one should analyze the maturity level of gonads by taking the samples of oocytes using canulator and observing the conditions and diameter of the oocytes after being mixed with serra solution. Table 2 showed that the highest levels of gonadal maturity that reached the final stage of gonadal maturation was 84.85% and the lowest was 45.71%. The final gonad maturation meant that 60-80% would successfully spawn after the administration of hormones. When the oocytes were ivory, their diameter was more uniform diameter and the egg size was larger, most of the oocytes could be separated/ were not coagulated[9,10].

Table 3. Oocyte Condition after being Mixed with Serra Solution

Fish	Oocyte Condition *	Description
1	A little coagulation, most of their sizes were not uniform	Poor
2	A little coagulation, most of their sizes were not uniform	Poor
3	No coagulation, size was uniform	Good
4	No coagulation, most of their sizes were not uniform	Poor
5	A little coagulation,	Poor

*Oocyte sample mixed with serra solution

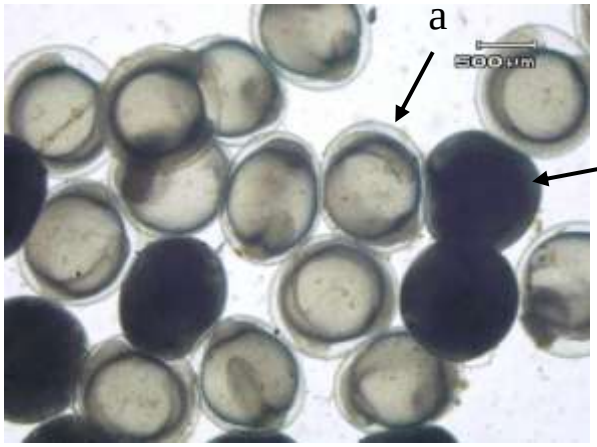


A **B**

A. The size was not uniform and a little bit coagulated
B. The size was not uniform and coagulated
Picture 2. Oocytes after being mixed with Serra Sollution

Influence of Fertilization Media pH towards the Degree of Fertilization and Hatching of P.hypophthalmus eggs

The degree of fertilization was determined once the eggs reached the gastrula stage that is by calculating number of fertilized and non-fertilized eggs



A. Fertilized eggs
B. Non-fertilized eggs
Gambar 3. P.hypophthalmus eggs after fertilization

The highest degree of P.hypophthalmus egg fertilization in fertilization media with different pH was in pH 8 (the percentage was 98.99%) and the lowest was in pH 6 (the percentage was 81.21%). In pH 7 treatment (control), the degree of fertilization was 90.78%. Based on Table 4, the degree of fertilization was high in the alkaline pH solution.

Table 4. P.hypophthalmus Eggs Degree of Fertilization in Fertilization Media with Different pH

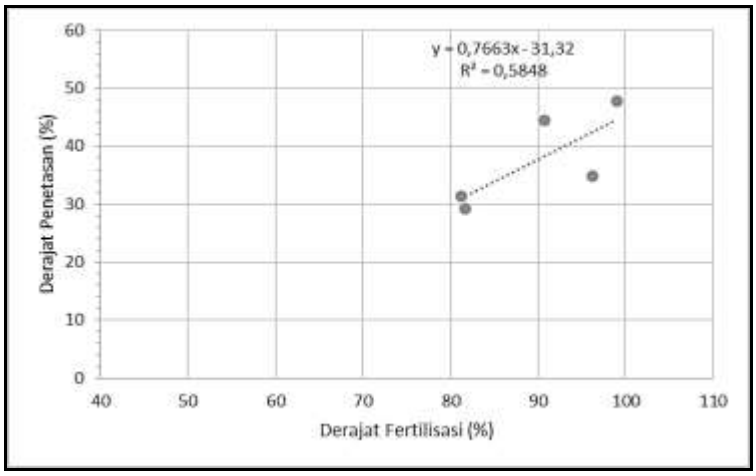
Treatment	Replication			Average
	1	2	3	
pH 6	82.35	70.97	90.32	81.21 ± 9.23[a]
pH 7 (Control)	90.91	91.43	90.00	90.78 ± 0.72[a]
pH 8	100	100	96.97	98.99 ± 1.75[c]
pH 9	94.59	100	94.12	96.24 ± 3.27[b]
pH 10	71.79	79.41	93.75	81.65 ± 11.15[a]

According to Billard[7], the optimum sperm motility of rainbow trout occured in pH 9. The optimum pH in the seminal plasma for the fertilization of rainbow trout was between 8.0 and 8.2.

Having studied the mechanisms of endocrinologt towards sperm motility in salmon, Nagahama[11] explained that gonadothropin induced somatic cells in the testicles, which induces the production of 17 ahydroxyprogesterone, then 17a, 20b-dihydroxy-4-pregnen-3-one was produced in spermatozoa, leading to an increase in the pH of the sperm ducts. The process in turn increases cAMP in the sperm and increased the sperm motility.

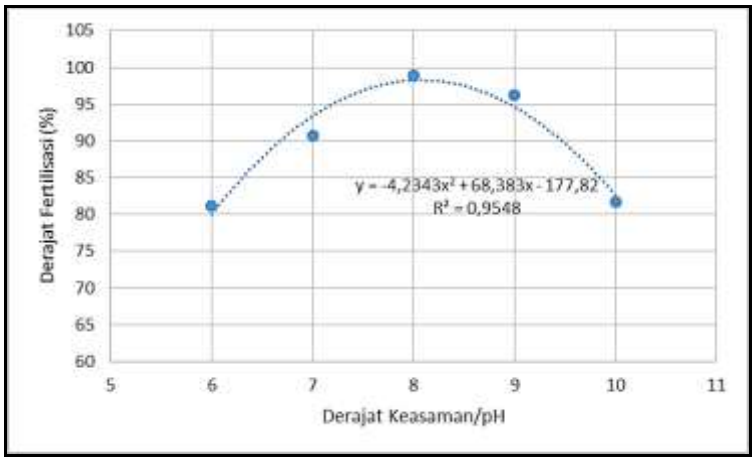
Table 5. P.hypophthalmus Eggs Degree of Hatching in Different Fertilized pH Media

Treatment	Replication			Average
	1	2	3	
pH 6	29.41	32.26	32.26	31.31 ± 1.65[a]
pH 7 (Control)	30.30	62.86	40.00	44.39 ± 16.72[a]
pH 8	32.26	62.50	48.48	47.75 ± 15.13[a]
pH 9	16.22	41.03	47.06	34.77 ± 16.35[a]
pH 10	20.51	29.41	37.50	29.14 ± 8.50[a]



Picture 4. Chart describing the Correlation between degree of fertilization and degree of hatching of P.hypophthalmus eggs.

The highest degree of hatching at pH 8 treatment was 47.75% that was directly proportional to the degree of fertilization, while the lowest degree of hatch ing at pH 10 treatment was 29.14%.



Picture 5. Chart describing the Correlation between pH and degree of fertilization of P.hypophthalmus eggs

Gallego et.al.[12] stated that degree of fertilization was directly proportional to puffer fish (*Takifugu niphobles*) degree of hatching.

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