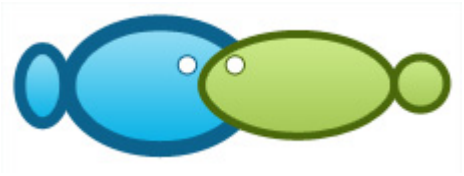

A preliminary observation on the effect of sperm extenders on the fertilization and hatching rates of seurukan fish (*Osteochilus vittatus*) eggs

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Abstract. The objective at the present study was to determine the suitable natural extender for seurukan fish (*Osteochilus vittatus*) sperm. Four extenders namely: young coconut water, old coconut water and sugarcane water were tested and physiological solution at three replicates were tested in this study. Sperms were diluted with respective extenders at ratio of 1:20 (sperm:exstenders). The diluted sperm were mixed with ovulated eggs of seurukan fish. A total of 100 eggs were selected randomly and about 2 mL of diluted sperm were added to the eggs in a beaker then mixed homogeneously then the eggs and sperm were left in contact for 5 minutes. Then the eggs were incubated in the plastic jar at water temperature of 28-29°C. The fertilization success was examined two hours after fertilization and the hatching rate was monitored six hours after fertilization at two hours interval. The data were tested by a one-way ANOVA and followed by a Duncan's multiple range tests. The Anova test showed that extenders gave a significant effect on the fertilization, hatching and survival rates of seurukan fish ($p < 0.05$). The best results were found at physiological solution, this value was different significantly with other extenders. Concerning in natural extenders that the young coconut water gave a good result compared to old coconut water and sugarcane water. In conclusion, the physiological solution is the best extender for seurukan fish spermatozoa.

Key Words: coconut water, sugarcane water, spermatozoa, fertilization, hatching rate.

Introduction. Seurukan fish (*Osteochilus vittatus*) is one of the indigenous higher economic value fish occurred in Aceh waters (Muchlisin 2013). Presently, the seurukan fish was cultured extensively or polycultured with other species such as common carp (*Cyprinus carpio*), tilapa *(Oreochomis niloticus*) or gouramy (*Osphronemus goramy*) resulting in lowing its productivity (Mulyasari et al 2010). One of the problems in seurukan fish culture is low hatching rate lead the farmers difficult to find higher quality larvae and in sufficient quantity. Therefore, the development of breeding technology of seurukan fish is crucially needed. The induced spawning of seurukan fish using some stimulating hormones has been reported by Muchlisin et al (2014), however, information about sperm extenders has not been previously studied.

Extender is a medium to dilute sperm and to get a larger amount of diluted sperm for artificial insemination for the breeding purposes. This is because fish produces high viscosity of sperm and in some cases only small volume is produced. Therefore, extenders are commonly used in artificial breeding of fishes in hatcheries, to induce sperm motility and increased fertilization rate (Muchlisin 2005). However, the suitability of extenders depends on fish species. In general, there are two types of extenders i.e. chemicals and natural extenders. The extenders must be isotonic to sperm to maintain electrolytes balance and osmotic pressure in sperm cells resulting a long lifetime of sperm (Ohta et al 2001).

The previous study on African catfish (*Clarias gariepinus*) showed that chemical extenders gave a best result compared to natural extender (Muchlisin et al 2010). However, chemical extenders had some disadvantages, for example toxic in higher

concentration of some diluted materials, costly and difficulties in finding some materials in remote areas hatchery, while the natural extender is cheap and easy to prepare, but resulted in low fertilization as recorded in some species for example African catfish (Muchlisin et al 2010; Muchlisin et al 2015). However, no studies have been conducted in extender exploration for seurukan fish. Hence, the objective of the present study was to evaluate the best sperm extender for seurukan fish sperm.

Material and Method

Experimental design. The experiment was conducted in Babah Krueng Hatchery Beutong, Nagan Raya district, Indonesia from 22 June to 6 July 2014. The completely random design was utilized in this study. The independent variable was type of extenders i.e. physiological solution, old coconut water, young coconut water, sugarcane water. The dependent variables were fertilization and hatching rate of seurukan fish eggs. The experiment was conducted in three replicates. The dilution ratio sperm:extender was 1:20 (v/v), this is the best ratio for African catfish (Muchlisin et al 2010) and bagrid catfish (*Mystus nemurus*) sperm (Muchlisin et al 2004).

Extenders. Fours extenders were examined i.e. old coconut water, young coconut water, sugarcane water and physiological solution. The old coconut water was collected from coconut with brown-darkness shell, while the young coconut water was collected from coconut with green shell and the flesh is soft. The cane water was obtained from old cane that has yellowish tree. According to Barlina et al (2007) the young coconut water had 5.20% sucrose and fructose, 81.80 mg L^{-1} magnesium, 730.40 mg L^{-1} potassium, these values change to 3.00%, 70.60 mg L^{-1} and 772.40 mg L^{-1}, respectively when the coconut reached old. The composition of extenders were presented in Table 1.

Table 1

The composition of minerals and sugars of the tested extenders

Compositions	*Young coconut water*	*Old coconut water*	*Sugarcane water*	*Physiological solution*
Sucrose/fructose (%)	5.20	3.00	15.05	-
Magnesium (mg L^{-1})	81.80	70.60	-	-
Potassium (mg L^{-1})	730.40	772.40	-	-
Sodium (mg L^{-1})	7.9	6.6	-	3538
pH	6.0	5.1	5.0	7.3

Induce spawning of brood fish. Two mature male fish (120 g and 125 g) and one mature female (160 g) were selected from a broodstock pond. The female and male broodstock were administered with single dose of Ovaprim of 0.5 mL kg^{-1} and 0.25 mL kg^{-1}, respectively, then the fish was reared in two different ponds, for male and female, respectively. Approximately six hours after hormone injection, the males were abdominal gently pressured and the sperm were collected with syringes then kept in the ice box (4°C). The collected sperm were mixed with the respective extenders at ratio of 1:20, sperm:extender (v/v) then kept in at temperature of 4°C in cruses ice box.

The ovulated female fish was abdominal gently pressured and the eggs were collected in a plastic basin at 4°C prior to use for fertilization test. A total of 100 eggs were selected randomly from the basin and about 2 mL of diluted sperm were added to the eggs in a beaker, then mixed homogeneously with a soft feather, then the eggs and sperm were left in contact for 5 minutes to allow fertilization process occurs. Then, the eggs were incubated in the plastic container at water temperature of 28-29°C. Every experiment was conducted in three replicates.

The fertilization success was examined four hours after incubation and the hatching rate was monitored six hours after incubation at two hours interval. Fertilization rate was evaluated by the development of transparent eyed embryos in contrast to an opaque white colour for unfertilized eggs (Muchlisin et al 2010). The survival rate of the

larvae was recorded seven days after hatched. The larvae were started to feed with daphnia (a zooplankton) three days after hatching.

Data analysis. The data of fertilization, hatching and survival rates were tested by a one-way ANOVA and followed by a Duncan's multiple range tests to determine if there were significant differences among treatments.

Results and Discussion. The Anova test showed that extenders gave a significant effect on the fertilization, hatching and survival rates of seurukan fish ($p < 0.05$). The best results were found at physiological solution, this value was significantly different with other extenders. Concerning the natural extenders, the young coconut water has given a better result compared to old coconut water and sugarcane water (Table 2). Therefore, young coconut water is possible to be used as natural extender for seurukan fish sperm. A similar study was reported in African catfish that the coconut water was also suitable for African catfish sperm, however the value was lower compared to Ringer solution (Muchlisin et al 2010). Therefore, this study and previous report showed that chemical extenders (Ringer and physiological solutions) are better compared to coconut water; however, in regard to natural extender the young coconut water shows a better result compared to other tested natural extender of sugarcane which was tested in this study and soybean milk which was reported by Muchlisin et al (2010).

Table 2

The average percentage (±SD) of fertilization, hatching and survival rates of seurukan fish eggs according to extenders

No.	*Extenders*	*Fertilization rate (%)*	*Hatching rate (%)*	*Survival rate at 7 days after hatch (%)*
1.	Physiological solution	95.00 ± 1.00^{d}	77.66 ± 6.50^{d}	76.66 ± 5.50^{c}
2.	Young coconut water	65.33 ± 3.05^{c}	45.33 ± 4.93^{c}	43.00 ± 6.08^{b}
3.	Old coconut water	51.00 ± 5.19^{a}	18.00 ± 3.0^{a}	16.00 ± 2.64^{a}
4.	Sugarcane water	57.33 ± 1.41^{b}	27.67 ± 2.51^{b}	24.33 ± 2.08^{a}

Values at same column with different superscripts are significantly different ($p < 0.05$).

The higher results at physiological solution probably due to Na^+ ions content in this solution was higher compared to young and old coconut water. The physiological solution has 3538 mg L^{-1} of Na^+, while the young and old coconut water have Na^+ ion of 7.9 mgL^{-1} and 6.6 mg L^{-1}, respectively (Barlina et al 2007; Arsa 2011), while no Na^+ ion in the sugarcane water (Chen & Chou 1993). The sodium acts as buffer in the medium which plays an important role in stabilizing of pH. The pH of physiological solution was 7.0 closed to pH of seurukan sperm of 7.4, while young and old coconut water, and sugarcane water have pH of 6.0, 5.1 and 5.0, respectively.

Generally the seminal plasma of fishes was ranged between 6.0-9.0 (Nahiduzzaman et al 2014; Islam & Akhter 2011; Perchec-Poupard et al 1997). Hence, the pH of extenders should be adjusted close to seminal plasma condition. This is in agreement with Suriyadi et al (2012) who stated that the physiological solution is isotonic to seminal plasma of fishes.

The lower concentration of sodium ions in old coconut water was also suspected to be the cause of low fertilization and hatching rates of seurukan eggs. According to Adipu et al (2011) the increases of K^+ content in medium will reduce the motility of sperms. In addition, Hamamah & Gatti (1998) reported that the presence of high potassium concentrations and low pH can inhibit sperm motility of fishes. While the fertility is strongly correlated to sperm motility (Muchlisin et al 2004). However, the inhibitory properties of potassium can be eliminated by the application of a sufficient concentration of calcium in the extender (Billard & Cosson 1992). The calcium acts as antagonistic with the inhibitory effect of potassium on sperm motility (Alavi & Cosson 2006). Hence, we suggest to add an appropriate concentration of calcium into the young coconut water to

minimize the inhibitory effect of potassium and therefore, the further study is needed to overcome this issue.

Conclusions. The higher fertilization and hatching rates were found in NaCl physiological solution, however in regard to natural extenders the young coconut water showed the best result compared to old coconut water and sugarcane water. Therefore, it is concluded that the young coconut water is suitable as an alternative extender of seurukan fish sperm.

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