Artificial spawning and larvae performance of three Indonesian mahseer species

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Abstract. This study attempts to evaluate the reproductive and larvae performance of the three species of mahseer in Indonesia through artificial propagation. The research was conducted at the Research Institute for Freshwater Aquaculture and Fisheries Extension, Bogor, Indonesia from August to December 2017. A completely randomized design was performed using three mahseer species (*Tor douronensis, Tor soro* and *Tor tambroides*) with three replications. The fecundity and egg diameter of *Tor douronensis* were (2.3±0.07 mm; 4987.80±17.25 eggs kg^{-1}), *Tor soro* (2.86±0.08 mm; 3157.02±97.28 eggs kg^{-1}) and *Tor tambroides* (3.00±0.06 mm; 1061.19±35.18 eggs kg^{-1}) respectively. The fertilization rate (FR) and hatching rate (HR) between species ranged between 76.00±7.21 and 93.33±1.15 (FR) and 80.36±6.00% to 93.32±4.57 (HR), while the survival rate of 15-day-old larvae was not significantly different between *Tor soro* and *Tor tambroides,* but significantly different with *Tor douronensis*.
Key Words: breeding, eggs, larvae, mahseer, reproduction.

Introduction. Four species of mahseer (genus *Tor*) were identified to be endangered, i.e., *Tor douronensis*, *Tor soro*, *Tor tambra* and *Tor tambroides* (Robert 1999), indigenous from Sumatra, Java and Kalimantan islands due to limited cultivation activity knowledge (Kunlapapuk & Kulabtong 2011) and environmental degradation (Asih et al 2008). Recognizing the importance of socio economic fisheries and protection of these natural stocks, the development of reproduction technology on aquaculture is necessary for sustainable production (Abderrazik et al 2016). The basic information on the potential of fish reproduction can be obtained from the gonadal maturation review until the fish spawn and produce larvae (Setyaningrum & Wibowo 2016).

Mahseer has been successfully domesticated in Indonesia. The breeding technology of *Tor soro* was declared successful in 2010 (Farastuti et al 2014). Indeed, the reproductive and larvae performance of mahseer needs to be developed to improve its production. Dunham (2004) states that fish from different locations have different reproductive characteristics related to the influence of interactions between their genetics and the environment. Generally, mahseer spawns naturally (Dinesh et al 2010, Redjeki 2007; Nautiyal 2014); however, Abderrazik et al (2016) conducted research on reproductive biology knowledge to determine the genetic and reproductive potential of *Sardina pilchardus*. A study on artificial spawning between existing mahseer species in Indonesia has never been done. The nutritional requirement and the condition of the environment both determine how broodstock is reared and maintained for gonad maturation. In this framework, the present study attempts to obtain baseline data related to reproductive performance and the larvae of three species of *Tor* produced by artificial spawning.

Material and Method. This reasearch was conducted from November 2017 to January 2018. Three species of *Tor* genus from the Research Institute for Freshwater Aquaculture and Fisheries Extension, (Bogor, Indonesia) were used as broodstock. The size of female broodstock ranged 40-62 cm total length (TL) and 940-4880 g in body weight (BW), while the male broodstock ranged 30-45 cm (TL) and 500-990 g in (BW). Five pairs of each species were acclimatized in ponds for approximately one month; they continued for gonad maturation, spawning and larvae rearing. Gonad maturation for each species was carried out by separately rearing between males and females in a 200 x 300 x 70 cm pond, carrying an artificial feeding containing 28% of protein with a 3% feeding rate per day (twice daily, morning and afternoon). Gonad maturation was measured every two weeks through the cannulation method.

The fish broodstock with mature gonads was injected with LHRH-a + anti-dopamine hormone at dosage 0.6 mL kg^{-1} of body weight (one injection at 13.00 pm and one at 17.00 pm, then stripped after a day). A gram of eggs was sampled and the egg number counted as the relative fecundity (eggs kg^{-1}) based on gravimetric analysis. Furthermore, the remaining eggs were fertilized with sperm and taken into the incubation in three aquariums with sizing 30 x 40 x 20 cm (500 eggs/aquarium). Each aquarium was equipped with the recirculating water system and a moderate aeration system. Each aquarium was stocked with 250 larvae fish from each species and was replicated three times. The larvae were reared without being fed until the yolk disappeared, but eventually fed at-satiation with *Artemia* nauplii (morning, afternoon and evening). Water quality management was done by water exchange (50%) using a siphoning method. The parameters measured consisted of fertilization rate (%), hatching rate (%) and abnormality (%) of the 15 days old larvae. The gonadal maturity was analyzed descriptively and the other parameters (egg diameters, time hatched, fertilization rate (FR), hatching rate (HR), abnormality and survival rate (SR)) were analyzed by ANOVA ($p < 0.05$). The water quality parameters including temperature, pH, dissolved oxygen (DO) and ammonia concentration were measured on day 0, 15, 30, 45 and 60 in-situ at 8 and 10 am.

Results. Data of the three broodstock mahseer, eggs and fish larvae is presented in Table 1.

Table 1

Data of three mahseer broodstock, eggs and their larvae

Parameter	*Tor douronensis*	*Tor soro*	*Tor tambroides*
Mature female (induce to ovulate)			
Total length / TL (mm)	405-435	460-520	516-600
Body weight / BW (g)	820-1020	930-1360	3580-4880
Number of female (individual)	5	5	5
Spawned fish (individual)	2	2	2
Atresia egg fish (individual)	-	1	2
Mature male			
Total length (mm)	355-450	299-318	300-342
Body weight (g)	460-990	290-300	300-390
Number of male	5	5	5
Spawned	5	5	4
Breeding			
Latency period (hour)	±18	±18	±16
Eggs			
Diameters of stripped eggs (mm)	2.3 ± 0.07^a	2.86 ± 0.08^b	3.00 ± 0.06^c
Total weight of stripped eggs (g)	40-50	50-70	70-100
Time hatched (day)	4.34 ± 0.03^a	5.02 ± 0.02^b	5.97 ± 0.02^c
Larvae			
Abnormality (%)	8.40 ± 1.83^a	2.47 ± 0.12^b	2.93 ± 0.31^b
Survival rate (%)	79.33 ± 0.95^a	82.11 ± 1.43^b	80.22 ± 1.05^b

Some data presented are averages of ±SD from three replicates. Different superscripts in the different columns show significant differences ($p< 0.05$).

Egg diameter. The average egg diameter among species at the time of spawning was 2.3±0.07 mm (*T. douronensis*), 2.86±0.08 mm (*T. soro*) and 3.00±0.06 mm (*T. tambroides*) (Figure 1). The egg diameter of the *T. douronensis* was smaller than others, while *T. soro* and *T. tambroides* were not different (Figure 2).

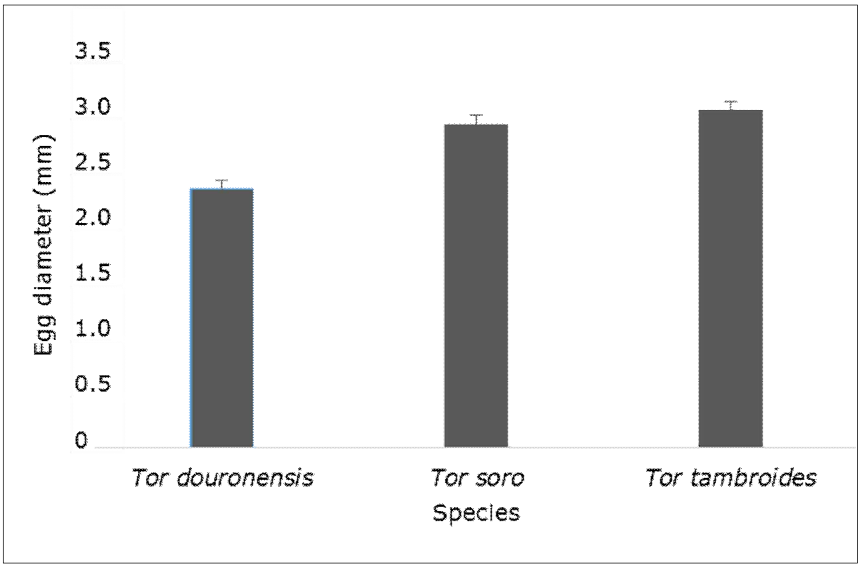

Figure 1. Average egg diameter of the three Indonesian mahseer species.

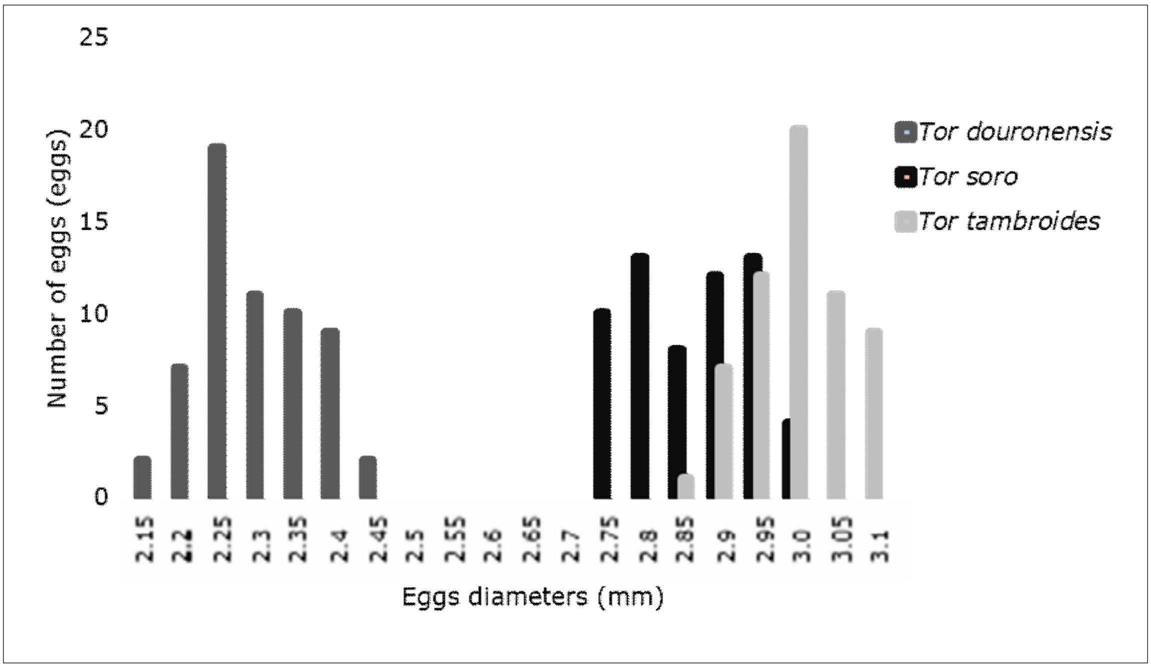

Figure 2. Distribution of egg diameters at the time spawning of the three Indonesian mahseer species.

Fecundity. The number of eggs (fecundity) among species was significantly different ($p < 0.05$), 4987.80±17.25 eggs kg^{-1} (*T. douronensis*), 3157.02±97.28 eggs kg^{-1} (*T. soro*) and 1061.19±35.18 eggs kg^{-1} (*T. tambroides*) respectively. The data refers to the weights and lengths of successful spawning broodstock.

Fertilization and hatching rates. The hatching and fertilization rates in the *Tor douronensis* were significantly lower than the other two species (Table 2). *Tor douronensis* had the shortest domestication period of the three. The values of the fertilization and hatching rates, in this study, are presented in Table 2.

Table 2

Fertilization and Hatching rates of three mahseer species

Species	*Fertilization rate*	*Hatching rate*
Tor douronensis	76.00 ± 7.21^a	80.36 ± 6.00^a
Tor soro	93.33 ± 1.15^b	93.32 ± 4.57^b
Tor tambroides	91.33 ± 1.15^b	90.20 ± 2.75^b

The data presented are averages±SD from three replicates. Different superscripts in the same columns show significant differences ($p < 0.05$).

***Analysis of water quality parameters**.* The water quality data during egg incubation and larval rearing is presented in Table 3.

Table 3

Water quality parameters of media for egg incubation and larval rearing

Water quality parameters	*Values*	
	Present study	*Other studies*
Water color	Clear	Clear*
pH	7.91-8.12	6.81-7.09**
Temperature (°C)	23.58-23.68	25.26-27.30**
DO (mg L^{-1})	7.16-7.30	6.30-8.34**
Nitrate (mg L^{-1})	-	1.46-3.10***
Nitrite (mg L^{-1})	-	0.004-0.2***
Ammonia (mg L^{-1})	0.08	-

(*) Kunlapapuk & Kulabtong (2011); (**) Rachmatika et al (2005); (***) Haryono & Subagja (2008).

Discussion. Feeding serves as a source of energy that will be used to sustain life, growth and the reproductive process. The time required for the process of gonad maturation up to spawning varies for each species, depending on the type of feed. The present study showed that mahseer fed with 28% protein of feed attained gonad maturity during 4-6 weeks. In this study, the gonad ripe response of *T. tambroides* was faster than the other two species. This was possibly due to the fact that *T. tambroides* has a relatively smaller protein requirement than *T. soro* and *T. douronensis*. According to Kamler (1992), protein is the dominant component in egg yolk, while the amount and composition of the protein is determined by the egg size. Mahseer fish, in the present study, showed natural changes in shape, through the differences in gonad maturation (abdomen). In general, the fish increased voluptuous gain, especially the inside part. However, the belly returned to its regular shape once the fish was ready for ovulation, marked by an increment volume and circumference of the abdominal cavity. A research conducted by Hardjamulia et al (1999) revealed that feeding of *T. douronensis* with 36% protein of feed led to the fish reaching gonad maturity within three weeks (21 days). According to Redjeki et al (1999) on the previous research, *Tor soro* fed with 35% protein of feed could reach maturity within 3-4 weeks and the abdominal cavity length of Kancra fish mature ranges 29.0–34.5 cm (Redjeki 2007).

Latency time of the *Tor* genus was between 16-18 hours after the second injection with water temperature 24-25°C. The latency duration was defined as the time between injection and stripping. During this period, fish ovulation was examined up to two times. The same dose of hormone 0.6 mL kg^{-1} was used, which caused the different fecundity and latency time. These results reinforce the assumption that the fewer eggs produced, the faster the latency time. Kristanto et al (2007) said that the range of latency time of mahseer fish (*T. soro*) from the last hormone injection was 12-19 hours, at water temperature 24-28°C; this latency time is necessary to know, because if it passes, the fish eggs will become atresia.

The broodstock were not all spawned in each species. The *T. tambroides* male and female spawned about 80%. It is suspected that this happened because the fish was sick, seen from its dark color and slow movement. The *T. douronensis* female was only 40%

successfully spawned, while *T. soro was* 60%. It was suspected that the nutritional requirement of females in each species was different, while the fish were given the same feed protein. Feed is an essential component needed for reproduction. The protein need was different for each species and generally ranges from 25 to 40%.

The egg diameter differences were allegedly caused by genetic (non-additive) and genetic-environment interaction (GE) factors. Each broodstock comes from a different environment (province). This is considered to affect the physical and reproductive fish. Falconer & Mackay (1996) stated that genotype by environment (G × E) interaction refers to the difference in the response of genotypes to different environments. In nature, the egg diameters of *T. douronensis* ranged from 2.22 to 2.49 mm (Gaffar et al 1991), while the egg diameters of *Labeobarbus douronensis* were 3.5±0.01334 mm (Redjeki et al 2001). The range of eggs' diameter varies, possibly for differences in gonad maturity and time required for the maturation process of each fish species. The difference is as a result of the process of vitellogenesis, hydration and grains formation of oil that runs gradually. The more uniform the egg diameter, the better the quality of spawning (Sivakumaran et al 2003). Different vitellogenesis processes are strongly suspected to be influenced by generalized feeding, whereas the nutrient needs of each species are not yet known.

The fecundity value of this type of fish (mahseer) still varied and could not be ascertained. For example, *T. tambroides* with an average broodstock body weight of 5200-8700 g produced 3125-8201 eggs at the time of spawning (Haryono 2006), while *T. douronensis* of 85.5 cm body length produced 63,360 eggs (Gaffar et al 1991) and those with a body length of 64.8 cm produced 14,433 eggs (Rupawan et al 1999). The fecundity of *T. soro* with the average body weight of 800-1000 g was 1752-2129 eggs (Farastuti et al 2014) and *T. tambroides* with a body weight of 2230-2850 grams had produced 1200-2800 eggs (Azuadi et al 2013). Gaffar et al (1991) mention *T. douronensis* fish in South Sumatra, which recorded 2073 eggs kg^{-1}. According to Bromage et al (1993), an important factor affecting eggs (number and size) is the size of the parent used. For the same age fish as in this study (2-3 year old): the greater the weight/size of the parent, the higher the value of fecundity. In total, the number of eggs produced is also in tune with the genetic diversity of each parent. The fecundity varied among the species and individual, and has been known to be dependent on brood conditions such as size (length, weight and age), genetics, food availability and environmental factors (Muchlisin et al 2011). The fecundity of large size fishes is linearly related to the total length of the fish (Joshi et al 2018).

At air temperatures of 23.58-23.68^{o}C *T. douronensis* hatched at the fastest (4.34±0.03 days), followed by *T. soro* (5.02±0.02 days) and *T. tambroides* (5.97±0.02 days). In general, *Tor* spp. hatch after 4-5 days of incubation at 25-27°C (Asih & Subagja 2003). Subagja et al (2013) stated that at a temperature of 25-26^{o}C, *T. douronensis* hatchery requires 72 hours with a hatching rate of 65%. Azuadi et al (2013) stated that the hatching rate of *T. tambroides* eggs was resolved between 57.8-82.5% at a temperature of 25-27^{o}C. The hatch time in this study is indeed slower, this is due to the lower incubation temperature. Even though the time needed to hatch is longer, but the HR and SR generated reach 90% more.

The fertilization rate in fish is largely determined by the quality of eggs, spermatozoa, media and human handling. Fish eggs will usually develop normally if the hatchery conditions include oxygen, temperature and pH are fulfilled. In addition hormones also give effect (Woynarovich & Horvath 1980). The use of hormones (sGnRHa + dopamine) not only encourages the female parent to ovulate alone, but also its decision with successful fertilization, hatching and larvae produced (I'tishom 2008).

The 15-days old survival rate (SR) showed no significant difference among species ($p > 0.05$). The survival rates of larvae *T. douronensis*, *T. soro* and *T. tambroides* were 79.33±0.95%, 82.11±1.43% and 80.22±1.05% respectively. This result was relevant with Subagja et al (2009); the maintenance of *T. douronensis* for 88 days had SR of 80%. The survival rate of *T. tambroides* ranged between 48.9 and 84.2% (Azuadi et al 2013). A high SR value can be ascertained if the number of larvae produced in mahseer is spawning quite well. So this type of fish is very good as aquaculture fish.

The bigest abnormality (8.40±1.83%) was observed on *T. douronensis*. This value was significantly different ($p > 0.05$) than that of the other two species. Many factors cause abnormality in fish larvae, including genetic factors on *Sparus aurata* (Fernandez et al 2008), nutrients deficiency such as phosphorus on *Melanogrammus aeglefinus*, vitamin C on *Oncorhynchus mykiss* and *Ictalurus punctatus*, vitamin A on *Sparus aurata*, vitamin K on *Melanogrammus aeglefinus* (Berillis 2015), heavy metal pollution on Nile tilapia (Hassanain et al 2012), water temperature on *Sparus aurata* (Georgakopoulou et al 2010) and sea bass *Dicentrarchus labrax* (Sfakianakis et al 2006) and a possible role of bacteria and parasites of many fishes in the Willamette River (Kent et al 2004).

In this study, the larvae abnormalities were suspected from genetic suits for eggs and larvae. Larvae are maintained in a controlled environment. Observation of abnormalities was done visually. Commonly acquired disorders were spinal defects such as kyphosis (bent upward), lordosis (crooked down) and scoliosis (crooked up and down / like S). Skeletal deformities are major factors that downgrade hatcheries production. The abnormality value obtained was relatively small; it was in line with Ismi et al (2007) where the percentage of abnormality was visually lower than that of the staining method, due to fish that visually looked normal, when they, in fact were defective. There is no much research on larval abnormalities in artificial spawning of mahseer. Yuliyanti et al (2016) researched on *T. tambroides* with different hatching temperature variations, showing a value between 2 and 10%.

The water quality has affected fertilization and hatching rate. In this study, the temperature was relatively lower than that in the natural conditions, although, the temperature was still suitable for the fish species. However, other water quality parameters exist in a good range for egg hatching and larval rearing. Management activities to maintain proper water quality are essential in egg hatching and larval rearing; a simple way is to use a recirculation system with a filter so that water quality remains stable.

Based on data, *Tor douronensis* has reproductive performance less good than the other two species. This is allegedly due to gonad maturity level in *Tor douronensis* at the lowest spawning time. The low level of gonad maturity in *Tor douronensis* is evidenced by the amount of fish that successfully spawn (two out of five individuals) and has the smallest egg diameters. Good level of gonadal maturity can be predicted from the number of eggs with a diameter above the average. Generally, the *T. soro* is the species with the best reproductive potential compared to the other two. This is evident from the uniformity of ovulated eggs size, FR, HR and SR.

The highest fecundity was in *T. douronensis,* so it was likely to produce many larvae, but had the lowest survival rate with the highest larval abnormality. The fish species that had high fecundity, high survival rate and low abnormality was *T. soro*. The egg diameter of the three species is related to fecundity; it was in accordance with the statement of Setyaningrum & Wibowo (2016) that the larger the egg diameter, the lower the fecundity. The size of the egg diameter affected the length of hatching time, where in the same hatching conditions, the eggs with a larger diameter hatch longer. The same egg diameter was obtained in *T. soro*, indicating that the species was the most ready to spawn and had good egg quality, because *T. soro* had adapted well to the cultivation environment compared to the other two species.

Conclusions. *Tor tambroides* was the species that had the latest gonad maturation with the highest egg diameter 3.00±0.06 mm and fecundity 1061.19±35.18 eggs kg^{-1}, while *T. douronensis* showed significantly lower on fertility and hatching rate with an average egg diameter of 2.3±0.07 mm and fecundity 4987.80±17.25 eggs kg^{-1}. However, the survival rate of 15-day-old larvae was not significantly different among species.

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