Controlling gonad maturation on snakehead (*Channa striata*, Bloch 1793) for eliminating impact of climate change

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**Abstract.** The consequences of climate change, in general, are changes in temperature and extreme season. Related to the fish gonad maturation, the effect of temperature can be distinguished by its effect on fish depending on the pattern of the reproduction and spawning season. In fish that spawn seasonally, rising temperatures in the tropical area signal the fish to ripen the gonads and migrate to the spawning area for ovulation in the early spawning season. Therefore, changing in the beginning and its duration of the season related to climate change will affect the maturity of fish gonads. In this paper communication, controlling gonad maturation on snakehead for eliminating impact of climate change is discussed. Observation of the gonad development on thirty mature snakehead stocks in Bogor, West Java, was conducted every month during a year. The obtained data were then compared to a similar previous study in the same area to have better insight on the impact of climate change on gonad development. To eliminate natural signal influence on the snakehead spawning, the study of gonad maturation in spawning (August to January) and out spawning season (February to July) using combination hormone of Pregnant Mare Serum Gonad (PMSG) and Anti Dopamine (AD) was done. The study used three breeders for each season, the dosage hormone of 2 mL/kg body weight was injected every month when the eggs were starting to develop, from September to November for spawning season and from May to July for out spawning season. The observed parameters were eggs diameter, fecundity, estradiol concentration, and gonadosomatic index. The results showed that reproductive performance tends to decline and fluctuate compared to the previous study done two years earlier. The highest eggs diameter, fecundity, and gonadosomatic index were in November. Hormone treatments in spawning and out spawning season were not significantly different (P>0.05) in the eggs diameter. The concentration of estradiol in the spawning season were higher than out spawning season, but they were not significantly different (P>0.05). Thus, hormone induction out the spawning season enabled to enhance breeder maturation as in the spawning season. Based on the result, hormone treatment could be used as an alternative to control maturation on the snakehead breeders from the impact of climate change.

1. **Introduction**
Snakehead is an important local fish in Indonesia [1]. In recent years, the price reaches Rp. 60,000/kg [2, 3]. The demand also increases due to the high albumin content in snakehead meat [4]. It is very useful for medical purposes, particularly for wound and cancer healing [5]. The highest consumption of fresh snakehead was in Central Kalimantan with 5.21 kg/capita and West Java for salted snakehead with 3,193 tons. This level of consumption and demand illustrated the size of the existing market in a region.
Aside from the economic aspect, snakehead has technical advantages such as fast growth and environmental stress tolerance due to the presence of additional breathing organs [6]. Basic studies of snakehead have been reported on population genetics [7], natural reproduction [8-10]. By 2015, the domestication technology of snakehead has been conducted [11], but not optimal yet due to the effect of season on the spawning activity. The consequences of climate change, in general, are changes in temperature and extreme season. Related to the fish gonad maturation, the effect of temperature can be distinguished by its effect on fish depending on the pattern of the reproduction and spawning season. In fish that spawn seasonally, rising temperatures in the tropical area signal the fish to ripen the gonads and migrate to the spawning area for ovulation in the early spawning season. Therefore, changing in the beginning and its duration of the season related to climate change will affect the maturity of fish gonads.

The high demand for snakehead fish has suppressed the population since the destruction of the habitat and the overfishing. Therefore, aquaculture effort was important to carry out since it can reduce the natural production dependencies. The main aspect of the success of domestication as an initial part of snakehead culture is the management of broodstock maturation [12].

According to Rottmann et al. [13], hormonal mechanism in fish affects the reproduction control system for maturation. The mechanism is generally controlled by the BHPG (brain - hypothalamus - pituitary - gonad) axis. FSH (Follicle Stimulating Hormone) is the hormone that plays important roles in gonadal maturation [14]. FSH hormonal application is ordinarily done with PMSG (Pregnant Mare Serum Gonad).

Hutagalung et al. [15] induced Pregnant Mare Serum Gonad and Anti Dopamine (PMSG + AD) in snakehead and showed that the best dose was 1.25 mL/kg with average value of IHS 1.37% and IGS 3.35% but no observations from the early maturity of the gonads. In the previous study, it was found that the use of PMSG + AD in 1.5 mL/kg fish snakehead can boost the gonad maturity compared with the lower dose treatment [16]. In Indonesia, snakehead spawning peak at once within a year (July - January) [8, 16].

This study identified natural reproduction performance within a year and the effect of induced hormone during out of the spawning season. The objectives of the study are to control gonad maturation through understanding natural bio-reproduction changes and hormonal manipulation on gonad maturation of snakehead catfish for eliminating the impact of climate change.

2. Methods

2.1 Natural reproduction experiment
Thirty mature stocks of snakehead were collected from open water in Parung District, West Java, Indonesia. They were all dissected to figure out the distribution of eggs diameter, fecundity and gonadosomatic index (GSI) for every month observation during a year. The obtained data were then compared to the similar previous study in the same area and analyzed relating to the climate change data recorded from the Indonesian Agency for Meteorology, Climatology, and Geophysics (BMKG).

2.2 Gonad maturation experiment in and out-spawning season
Induce of combination hormone of PMSG and AD with the dosage of 2 mL/kg body weight was performed in-spawning season (August - January) and out-spawning season (February - July) using three breeders for each season as replication. Hormone treatments were given when eggs starting to develop, injected every 30 days from September to November in-spawning season. Out-spawning season, hormone injection was given form May to July. The parameters observed in this experiment were the development of egg diameter before and after hormone treatment and the estradiol-17β concentration.
3. Results
Observation of natural reproduction performance in a year, hormone induction to enhance egg maturation, breeder maturity during out of spawning season are presented below.

3.1 Natural reproduction experiment
Reproductive performance of snakehead tends to decline and fluctuate compared to the previous study done two years earlier. The highest eggs diameter, fecundity, and GSI were in November (Table 1). The data of temperature changes and rainfall showed that egg diameters, fecundity and gonadosomatic index were high at moderate temperature changes and high rainfall (Figure 1).

Data and information on natural reproduction in fish are very important to understand since the success of hormone manipulation on fish breeding depends on the optimal condition of target fish. The results showed that the highest egg diameter, fecundity and GSI were in November and December. Three observed biological parameters were congruent, and they were optimal during November and December.

Based on the observation of reproduction pattern of snakehead in nature, hormone induction for broodstock maturation in the spawning season can be carried out since August before the size of egg diameter reaches the peak phase. Outside of the spawning season, the hormonal induction should be performed with a longer period after the atresia and before eggs begin to develop.

Table 1. Comparison on egg diameter, fecundity, and gonadosomatic index of *Channa striata* at 2016* and 2018

| Month    | Egg diameter | Fecundity | GSI   |
|----------|--------------|-----------|-------|
|          | 2016   | 2018   | 2016 | 2018 | 2016 | 2018 |
| July     | 0.9 ± 0.12 | 0.8 ± 0.06 | 3035.4 ± 10.38 | 3026 ± 20 | 2.8 ± 0.04 | 2.91 ± 0.045 |
| August   | 1.2 ± 0.07 | 0.9 ± 0.1  | 3051.8 ± 6.94  | 3604 ± 31 | 2.8 ± 0.05 | 2.71 ± 0.012 |
| September| 1.3 ± 0.07 | 0.9 ± 0.03 | 3060 ± 7.91   | 3100 ± 19 | 2.9 ± 0.07 | 2.88 ± 0.083 |
| October  | 1.4 ± 0.05 | 1.1 ± 0.05 | 3067.6 ± 5.59 | 4239 ± 42 | 3 ± 0.05   | 2.96 ± 0.051 |
| **November** | **1.5 ± 0.04** | **1.5 ± 0.04** | **3070 ± 3.81** | **4600 ± 35** | **3.2 ± 0.08** | **2.98 ± 0.031** |
| December | 1.5 ± 0.04 | 1.3 ± 0.02 | 3070 ± 7.91   | 4690 ± 29 | 3.3 ± 0.09 | 3.1 ± 0.013 |
| January  | 1.4 ± 0.09 | 0.9 ± 0.01 | 3080 ± 7.91   | 3584 ± 24 | 3.3 ± 0.05 | 2.81 ± 0.056 |
| February | 1.3 ± 0.07 | 0.8 ± 0.1  | 3067 ± 4.69   | 3421 ± 30 | 2.9 ± 0.11 | 2.83 ± 0.071 |
| March    | 1.1 ± 0.12 | 0.7 ± 0.02 | 3057 ± 4.69   | 3050 ± 45 | 2.5 ± 0.07 | 2.76 ± 0.027 |
| April    | 1.1 ± 0.11 | 0.6 ± 0.09 | 3055 ± 3.78   | 3182 ± 51 | 2.5 ± 0.07 | 2.79 ± 0.074 |
| May      | 0.9 ± 0.25 | 0.6 ± 0.07 | 3045.8 ± 5.12 | 3406 ± 63 | 2.3 ± 0.16 | 2.75 ± 0.053 |
| June     | 1.0 ± 0.16 | 0.7 ± 0.02 | 3047 ± 4.69   | 2930 ± 48 | 2.8 ± 0.06 | 2.71 ± 0.047 |

*Source: Bijaksana [9]
3.2 Gonad maturation experiment in and out-spawning season

Induction of 2 mL/kg body weight of hormone in the spawning season (August-January) and out the spawning season (February-July) was no significant difference in eggs diameter development (P>0.05) (Table 2). Thus, hormone induction out the spawning season enabled to enhance breeder maturation as in the spawning season. Even though, the concentration of estradiol in the spawning season were higher than out spawning season, but they were not significant difference (P>0.05) (Figure 2).
Table 2. Egg diameter of *Channa striata* breeders resulted from hormone injection in and out spawning season

| Induction period                  | Initial egg diameter (mm) | Final egg diameter (mm) | ∆ Egg diameter l (mm) |
|----------------------------------|---------------------------|-------------------------|----------------------|
| (August – January) Spawning Season | 0.47±0.021                | 1.45±0.023              | 0.98±0.015           |
| (February – July) Out spawning season | 0.56±0.033                | 1.43±0.010              | 0.87±0.025           |

Figure 2. Estradiol concentration in blood of *Channa striata* breeders resulted from hormone injection in and out spawning season

4. Discussion

Ex-situ reproductive performance under controlled environment is one of the major factors in the success of domestication [18, 19]. According to Zohar and Mylonas [20], in-situ environment has an advantage for optimal reproduction performance due to hormonal trigger available from natural condition. Since hormone manipulation on fish developed, many efforts have been done to control fish reproduction out of the natural habitat and season. To optimize hormonal treatment given on the target fish, data, and information on natural reproduction are needed [21, 22].

The information mainly relates to the gonad maturation stage and the peak of spawning time throughout the year. In Indonesia (Java, Sumatra, Kalimantan, and Sulawesi), it was reported that throughout the year, various gonad maturation stages of snakehead exist [8, 23-25]. It was assumed that snakehead were able to spawn throughout the year. However, based on the observation of egg diameter, fecundity, and GSI as an indicator of reproduction status, the peak season of optimum gonad maturation in nature was occurred once within a year [16]. Saputra et al. [10] support the previous
study that the peak of snakehead spawning in nature occurs in rainy season. The present study also showed that the spawning season in nature occurred during rainy season in October, November, and December indicated by optimum size of egg diameter, highest fecundity, and GSI.

Based on the data and information of natural gonad maturation, the suitable time to do hormone treatment for maturation is several months before spawning season starting from October every year. The proper understanding of reproductive cycle could be very useful in managing gonad maturation of fish and controlling the mass spawning for fry production. Using the effective hormone and the optimum dosage, the spawning of breeders can be managed periodically. Theoretically, the reproductive hormone mechanism of fish in the spawning season is controlled by the brain - the hypothalamus-pituitary-gonad axis [13, 21]. Environmental signals such as rain, temperature, media are received by the brain as central nervous system and forwarded to the hypothalamus [22, 26, 27]. The hypothalamus responds by releasing Gonadotropin-Releasing Hormone (GnRH) and dopamine that affecting pituitary gland. Furthermore, Gonadotropin Hormone which is containing Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) acting on gonad as target organs. Hormonal induction for gonad maturation is mostly done with PMSG (Pregnant Mare's Gonadotropin Serum). This hormone contains Follicle Stimulating Hormone (FSH), which plays a vital role in early gonad maturation or vitellogenesis [13, 21, 28]. The absorption of vitellogenin increase the oocyte size for ovulation. The application of PMSG has succeeded stimulate follicle to produce estradiol-17β in zebrafish *Oryzias latipes* [29]. In addition to the PMSG function in gonad maturation, Anti Dopamine (AD) has also an important role in inhibiting the dopamine mechanisms [30-32]. When the dopamine activity is blocked with Anti Dopamine, the role of dopamine is stalled, and the secretion of gonadotropin will increase [20, 28, 33]. In the current study, the combination of PMSG and AD hormone were used for snakehead gonad maturation. Study on this hormone combination has been conducted on climbing perch *Anabas testudineus* [34], Indonesian mahseer *Tor soro* [35], striped catfish *Pangasius hypophthalmus* [36, 37], leopard coral grouper *Plectropomus leopardus* [38], Indonesian shortfin eel *Anguilla bicolor* [39], African catfish *Clarias* sp. [40], red fin shark fish *Epalzeorhynchos frenatus* [41]. Induction of PMSG and AD hormone for snakehead gonad maturation has also been studied. Hutagalung *et al.* [15] study indicated that the induction of 1.25 mL/kg of PMSG + AD (Odev) gives the best value on IHS and IGS. However, hormone induction was not performed from the initial phase of gonad maturity, and observations were not conducted periodically. The results of this study showed that the dose of PMSG + AD 2 mL/kg body weight gave the best results in the final egg diameter, and the concentration of estradiol-17β. The egg diameter and biochemical components in blood plasma, including estradiol-17β, can be used to predict sexual maturity in female fish [42]. Compare to the previous study done by Ath-thar *et al.* [17] using the dose PMSG + AD of 1.5 mL/kg body weight, higher dosage in the present study gave better final egg diameter.

In commercial aquaculture, the availability of seed throughout the year is a limiting factor to support the continuity of snakehead production. Several efforts to solve seasonal spawning on some species have been done under controlled or manipulated environment [43, 44]. On snakehead, the appropriate manipulations of the external environment, such as fluctuating water depth in pond is able to trigger breeders to spawn out of season [9]. In the temperate zone, some cultured species are triggered on photoperiod to make them spawn (45-48).

In general, objectives of manipulation treated is to initiate environmental cue to generate signals for stimulating gonad maturation. Due to the absence of environmental cues in out of spawning season, technology of hormone induction enables be used for gonad maturation. It may influence the neuroendocrine components for gonad development [22, 49]. So far, there is no research that has been conducted on the gonad maturation of snakehead using hormone induction out of the spawning season. The result of current study working on the hormonal treatment of breeders snakehead during spawning and out spawning season showed there was no significant difference in gonad maturation performance. It suggests that hormone induction enables to influence the gonad maturation even though there is no stimulation from the environmental cues. In this case, the induction of combination of PMSG and AD hormones worked effectively for gonad maturation. According to Cardinaletti *et al.* [22], the gonad maturation was influenced by the presence of PMSG that contains FSH, which plays a vital role in
early gonad maturation or vitellogenesis. They mentioned that the general scheme of vitellogenesis were the sequent step as hepatic synthesis of vitellogenin, the delivery of vitellogenin via the maternal circulation, and the selective uptake of vitellogenin and cytoplasmic translocation of vitellogenin to form yolk bodies.

Through a comprehensive study, it can be concluded that the success of induced-breeding program needs the right time to apply hormone treatment, suitable hormone, and the effective dosage is given. Even more, we are also able to do those of practical technology to breed snakehead during out of spawning season.

5. Conclusion
Egg diameters, fecundity, and gonadosomatic index were high at moderate temperature changes and high rainfall in November and December. Hormone induction out the spawning season enabled to enhance breeder maturation as in the spawning season. Based on the result, hormone treatment could be used as an alternative to control maturation on the snakehead breeders from the impact of climate change. Further study on the level of higher dosage hormone of the same used in the present is needed to be tested on various sizes of the breeders. Another analog hormone is also possible to be tested to have an alternative for the economic price one.

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