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# Improvement reproductive performance of female *Betta channoides* in ex situ-rearing with maturation hormonal therapy

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**Abstract**
The goal of this research is to find the best dose of Oodev for enhancing female *Betta channoides* ex-situ reproductive performance. Four pairs of broodstock were acquired from a wild habitat in East Kalimantan, Indonesia, with total lengths of 4-5 cm and body weights of 0.6-0.9 g. The treatment was the delivery of Oodev maturation hormone to the broodstock by enrichment in *Moina* sp. as live feed at various doses, namely: without Oodev (control/$T_0$), enriched with Oodev doses of 0.3 ml/l ($T_1$), 0.6 ml ($T_2$), and 0.9 ml ($T_3$). Inducing the Oodev hormone in *B. channoides* for 90 days of ex-situ maintenance using the oral method trough live feed enrichment was successful and consistently improved over the control. We evaluated utilizing 0.6 ml/L dose of Oodev as the applicate optimal dose with the highest total larvae generated and most frequent spawning (4 times in 90 days of rearing).

**Keywords:** Fish species, conservation, broodstock, feed enrichment, spawning

## 1. Introduction
*Betta channoides* is a species of wild B. fish belonging to the *B. albimarginata* group which is found around the upper reaches of the Mahakam River, East Kalimantan, Indonesia (Kottelat & Ng 2005) [10]. The International Union for Conservation of Nature (IUCN) Redlist has categorized *B. channoides* as an endangered species because of a lack of populations in wild (Low 2019). Even though *B. channoides* are considered an endangered species of fish, commerce still occurs. This has a significant impact on the capturing in its natural habitat, which might deteriorate the situation affecting the wild population. The International *Betta* Congress (IBC) has grouped *B. channoides* into the wild *Betta*–small mouthbrooder category in ornamental *Betta* contests (Permana *et al*. 2021) [17]. So that *B. channoides* has been exclusively bred by hobbyists who have indirectly created ex-situ conservation. In the species' recovery campaign, the ex situ population has been crucial as a source for reintroduction and as a reservoir for preserving genetic diversity (Gautschi *et al*. 2003) [8].
Gonad maturation, ovulation and spawning of fish are related to hormonal control on the brain-hypothalamus-pituitary-gonad axis (Nagahama 1994) [14]. Environmental signals influence the reproduction of various species in the wild. For instance, seasonal floods and changes in flow rates are identified as spawning triggers for many fish species (Baumgartner *et al*. 2014; Jenney *et al*. 2022) [4, 9]. Due to the absence of environmental variables that drive the development and maturity of the gonads until ovulation and spawning, some fish cannot reproduce optimally in captivity (Mylonas *et al*. 2010) [13]. Therefore, the use of exogenous hormones is an effective way to stimulate gonadal maturation in fish.
Oodev (IPB University, Indonesia) is one of the commercial hormones widely utilized to induce gonad maturation in fish. The ingredient PMSG+DA (pregnant mare serum gonadotropine + dopamine antagonist) found in Oodev might trigger an increase in GnRH (gonadotropine releasing hormone) levels, which in turn promotes the pituitary to produce gonadotropine that stimulate the ovarian maturity in the fish (Nainggolan *et al*. 2014) [15].

Oodev successfully enhances gonadal maturation in Asian redtail catfish (*Hemibagrus nemurus*) (Putri *et al*. 2019) [18], catfish (*Pangasius hypophthalmus*) (Agustinus 2013) [1], *Poropuntius tawarensis* (Mellisa *et al*. 2022) [12], and snakehead (*Channa striata*) (Anwar *et al*. 2018) [3]. However, no reports of Oodev being used to accelerate *B. channoides* gonadal maturation exist. In this study, we were encapsulated Oodev to the live feed (zooplankton *Moina* sp.) to increase the reproductive performance of female *B. channoides* by oral application. This study also aims to determine the optimal dose of Oodev in increasing *B. channoides* ex-situ reproductive performance.

## 2. Material and Methods

This research was conducted on Januari until November 2019 at Ornamental Fish Culture Research Center, Depok, West Java, Indonesia. The research was carried out in accordance with Republic of Indonesia Law No. 18 of 2002 on the National System of Research, Development, and Application of Science and Technology. The research was conducted with the approval of the Research Institute for Ornamental Fish and in line with ethical principles (Letter of Assignment from the Head of the Research Institute 136/BRSDM-BRBIH/HK.060/I/2019).

### 2.1 Fish origin and rearing

Totally four pairs *B. channoides* broodstock with total length 4-5 cm and body weigth 0.6-0.9 g was obtained from wild habitat in East Kalimantan, Indonesia. The broodfish had been adapted and bred in captivity. The broodfish was reared in pair in a plastic container (40 x 25 x 20 $cm^3$) with a water level of 15 cm, stagnan, and given low aeration. A PVC (polyvinyl chloride) pipe with a diameter of 10 cm and a length of 10 cm was placed inside and used as a shelter. A total two dried catappa leaves were given in media to maintain optimal soft blackwater conditions and to lower the pH of the media according to the conditions of its wild habitat. During rearing, fish were fed *Moina* sp. once a day adlibitum. To maintain water quality, a total water change was carried out every two weeks with settled water.

### 2.2 Experimental design and data analyses

This study was designed with four treatments (no replication). The treatment in this study was the administration of Oodev gonadal maturation hormone to the broodstock through enrichment in *Moina* sp. as live feed with different doses, namely: without Oodev (control/$T_0$), enriched with Oodev doses of 0.3 ml/l ($T_1$), 0.6 ml ($T_2$), and 0.9 ml ($T_3$).

*Moina* sp., which contains the Oodev hormone, was fed to the fish once a week for three months according the treatment. *Moina* sp. enrichment was carried out for six hours in one liter (with density 100 *Moina* sp./ml) of aerated fresh water mixed with Oodev hormone at the dose according to the treatment design. After six hours, the *Moina* sp. were harvested and given to the fish according to the treatment. The dissolved oxygen, temperature, pH, Ammonia ($NH_3$), and nitrite ($NO_2$) was measured during experiment at 5.05-6.23 ppm, 27-30 ℃, 4.72-6.50, 0.03-0.05 ppm, and 0.02-0.04 ppm.

The parameters were observed in this study are average of total larvae per spawning (larvae/spawning), spawning frequency (Time), average of rematuration period (days), and Gonado somatic index (GSI, %). GSI was determined following formula

GSI (%) = (GW/BW) x 100%

Where GW is gonad weight (g), BW is body weight (g). All data were analyzed descriptively.

## 3 Results

Table 1 shows the reproductive performance of female B. chanoides in ex situ-rearing for 90 days with maturation hormonal therapy via oral application trouhght *Moina* sp. enrichment. Based on the data we know that the highest spawning frequency, gonado somatic index, total larvae per spawning, and the fastest rematuration period were occurs in 0.6 ml/l Oodev treatment with a value of 4 time, 3.35±0.58 %, 25±6 larvae/spawning, and 22 days. The lowest spawning frequency, gonado somatic index, total larvae per spawning, and the slowest rematuration period were occurs in 0 ml/l Oodev treatment (control) with a value of 2 time, 3.17±0.32 %, 22±2 larvae/spawning, and 35 days.

**Table 1:** Reproductive performance of female *B. chanoides* in ex situ-rearing for 90 days with maturation hormonal therapy using oral application trouhgh *Moina* sp. enrichment.

| Parameter | Treatment of Oodev dosage (ml/L) | | | |
|---|---|---|---|---|
| | **0** | **0.3** | **0.6** | **0.9** |
| Average of total larvae per spawning (larvae/spawning) | 22±2 | 24±4 | 25±6 | 24±5 |
| Spawning frequency (time) | 2 | 3 | 4 | 3 |
| Average of rematuration period (days) | 35 | 30 | 22 | 27 |
| Gonado somatic index (%) | 3.17±0.32 | 3,03±0.08 | 3.35±0.58 | 3.34±0.56 |

## 4 Discussion

Inducing the Oodev hormone in *B. channoides* for 90 days of ex-situ maintenance utilizing the oral approach trough live feed enrichment was successful in all treatments and consistently improved to the control. The hormone Oodev is a combination of domperidone (dopamine antagonist) and pregnant mare serum gonadotropin (PMSG) (Mellisa *et al*. 2022) [12]. PMSG is a glycoprotein hormone that has both luteinizing (LH) and follicle-stimulating hormone (FSH) activities (Christakos & Bahl 1979) [7]. FSH is involved with the beginning of gonadal maturation or vitellogenesis, whereas LH is involved ovulation (Palermo 2007) [16]. Domperidone can reduce pituitary LH secretion by suppressing dopamine (Anderson *et al*. 2013) [2]. Due to some hormone contain, the Oodev treatment can increase total larvae pers pawning and accelerate gonadal maturity afterwards spawning, as seen by a faster maturation time and followed by more often spawning during the treatment.

GSI improves in Oodev treatments dosage 0.6 ml/l and 0.9 m/l compared to controls due to FSH contain in Oodev. FSH is involved vitellogenesis (vitellogenin produced) (Palermo 2007) [16]. Vitelogenin was generated in the liver, transported by blood to the oocytes, then preserved as yolk, resulting in increased egg size, ovary weight rise, and an increase in GSI (Çek *et al*. 2001) [6]. The GSI technique could be used to identify mature ovaries in a variety of tiny, multiple-spawning

species of fish (Brewer *et al*. 2008) [5]. The GSI value in the current study was below 20 %. As a result, *B. channoides* appear to belong to the classification of fish that have low GSI values and can be classed as fish that can reproduce numerous times every year (Mellisa *et al*. 2022) [12]. The gonadosomatic index (GSI) is the percentage of a value determined by comparing the weight of the gonads to fish body weight (Brewer *et al*. 2008) [5]. The size and weight of the gonads produced will have achieved their maximum till right before spawning (Mellisa *et al*. 2022) [12]. As applicate optimum dose, we considered using 0.6 ml/L dose of Oodev with higher total larvae produced and the most frequent spawning (four time in 90 days of rearing).

## 5. Conclusion

The oral approach of generating the Oodev hormone in *B. channoides* for 90 days of ex-situ maintenance via live feed enrichment was successful in all treatments and consistently improved over the control. We tested a 0.6 ml/L dose of Oodev as the applicate optimum dose with the greatest total larvae produced and the most frequent spawning (4 times in 90 days of rearing).

## 6. Acknowledgement

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