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SKRETTING

Could domperidone via oral administration enhance final oocyte maturation and ovulation and in the long-term affect egg and larval quality in sand bass *Psammoperca waigiensis*?

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Dopamine is a neurotransmitter that inhibits the release of hormones from the pituitary. In the teleost fish brain, hypothalamic factors are released and transported to the anterior pituitary via the hypothalamo-pituitary portal system (Finn-Arne Weltzien *at al*., 2005). Under natural conditions, there is a feedback mechanism in the fish that limits the release of gonadotropin (GtH). This mechanism uses dopamine, which inhibits the action of gonadotropin releasing hormones (GnRH). When dopamine is present in the fish, even GnRHa will have only limited success (Jeff Mittelmark et al., 2006). Domperidone (DOM) is a dopamine antagonist (DA) that blocks the action of dopamine. When GnRHa and a dopamine antagonist are used in combination, reproductive success dramatically increases (Jeff Mittelmark *et al*., 2006). The inhibitory effect of dopamine on GtH secretion and on GnGH-induced GtH release is well established in most freshwater fish species (Nguyen Tuong Anh., 1998). In Vietnam, beside the freshwater fish, several marine finfish species, spawning under captivity conditions can be induced by environmental stimulus or hormone injection in combination with a dopamine antagonist (Nguyen Tuong Anh., 1998). DOM is the dopamine antagonist that has commonly been used for cultivated fish and proven to be very effective and reliable in induced spawning cultivated freshwater fish (Naruepon Sukumasavin *et al.* 2000).

Sand bass (*Psammoperca waigiensis*) is a tropical marine finfish present in coastal waters in Asia Pacific such as Vietnam, Indonesia, Singapore, Thailand, Japan and Australia (Tamaki Shimose *et al*., 2006). In Vietnam, induced spawning with hormone

Figure 1. Selecting breeders.

injections and environmental stimulations has been achieved in captivity (Nguyen Trong Nho *et al.*, 2003). For the commercial purpose of sand bass fry production, injection of GnGH analog combined with DOM was recommended rather than inducing with environmental stimulus (Nguyen Trong Nho *et al.*, 2003). The injections might, however, cause stress to fish, affect health and in the long-term influence egg and larval quality (Kjørsvik *et al.*, 1990). Therefore, an alternative method of using the dopamine antagonist, DOM, via oral administration to minimize the injections has been tested. In the present study, we investigated whether DOM via oral administration could enhance final oocyte maturation and ovulation (FOMO), and long-term effect egg and larval quality in sand bass.

Materials and methods

The experiments were conducted in a backyard-shrimp hatchery at Nha Trang City, Vietnam from February to August 2007. Three year old brood fish, hatched in late 2004, were kept in the ponds in which the salinity ranged from 28-32 ppt and temperature from 28-32°C before the experiments. On February 19, 2007, four groups of 60 fish (sex ratio is 1:1) were distributed in concrete tanks. The holding density was 3 kg broodfish per cubic meter. Broodfish were daily fed of 3-5 % body weight with trash fish and once per week with squid. Vitamin E and C were also supplied once per week in order to help stimulation the gonadal development and maturation (Syamsul Akbar, *et al.*, 2005). Domperidone was ground and mixed with cassava powder and finally with trash feed to form a paste. Fish were fed DOM every 3 days. Water temperature during the experiments was maintained of 28-32°C and water exchange of 100 % was taken place twice per week.

The fish were anaesthetized in cold freshwater to measure weight, length and for stripping. Once each month fish were weighed to the nearest 0.1 g and measured to the nearest 0.1 cm in length. Females and males were anaesthetized to check for maturity every two weeks. No hormone manipulation was applied to induce spawning in this study. The fish were induced to spawn by adding water to simulate the rising of the tide. This water change also drastically brought down the temperature to 27 or 28°C. Absolute fecundity (FA) was calculated from counting of egg samples (0.5-1 g) at stage IV (complete yolk formation), and relative fecundity (FR) calculated from: FR=100 FA. W -1 where FA is absolute fecundity and W is total weight of the female. Egg diameter (right after stripping) was determined as the average from measurements of 50 eggs under microscope equipped with micrometer. Fertilization rate was estimated by examining at least 50 eggs at the 32-cell stage. Eggs were cleared in a solution of glacial acetic acid and saline (1: 20 v/v), examined under stereomicroscope and cleaved eggs were classified as fertilized (Tveiten *et al.*, 2001). Gonad samples from the posterior, middle and anterior part of

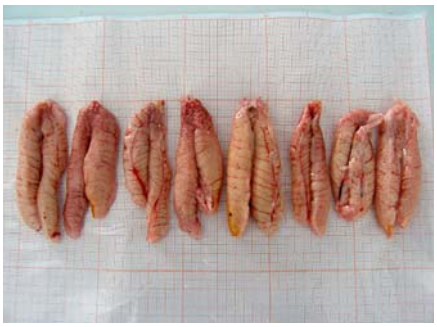

Figure 2. Ovaries from different groups have the same developed stages (complete yolk formation).

the right gonad were fixed in Bouin's fixative, dehydrated through an ethanol series and embedded in paraffin. The sections were cut at 4 - 6 um thickness and stained with Harris' Hematoxylin and Eosin for examination at light microscopy.

Eggs were considered normal when cleavage was symmetrical, cells had similar size and cell formation was complete, whereas abnormal eggs were associated with irregular cleavage, poor cell formation with vesicular inclusions, and deformation of blastomeres (Kjørsvik *et al.*, 1990). The proportion of eggs, which survived to the eyed stage, and until hatch, were assessed relative to the number of fertilized eggs. Hatching rate was possible to assess at the group level only. Hatching time was determined as the number of days from fertilization until 50% of the eggs were hatched. The effect of different DOM concentrations on the ovulating females, fecundity, egg diameter and embryonic development parameters

Table 1: Reproductive characteristics of female sand bass fed DOM during the breeding season.

Group	Weight (g)	Length (cm)	Absolute fecundity	Relative fecundity (egg kg^{-1})	Egg diameter (µm)	n
05 mg DOM /kg BW	313.5 ± 3.2	26.3 ± 2.5	112,250 ± 230	326 ± 3.4	756 ± 25	11
10 mg DOM /kg BW	325.2 ± 2.6	27.2 ± 3.7	106,421 ± 264	371 ± 4.5	740 ± 28	12
15 mg DOM /kg BW	291.7 ± 4.5	24.6 ± 4.3	98,160 ± 255	321 ± 6.5	755 ± 31	10
Control (no DOM)	296.7 ± 2.5	26.6 ± 3.3	100,160 ± 198	341 ± 6.8	735 ± 31	15

Table 2: Egg and larval quality of female sand bass fed DOM during the breeding season.

Parameters of egg quality	Group			
	05mg DOM/kg	10 mg DOM/kg	15mg DOM/kg	Control (no DOM)
Fertilization rate (%)	67 ± 3.2	65 ± 2.4	55 ± 2.2	70 ± 2.5
Survival to the eyed stage (%)	62 ± 2.7	58 ± 2.2	51 ± 2.5	70 ± 4.2
Hatching rate (%)	40 ± 3.6	45 ± 2.3	38 ± 2.6	49 ± 3.3
Survival to finished yolk sac stage (%)	26 ± 4.6	23 ± 3.3	22 ± 5.6	33 ± 4.3
Embryonic development duration (h)	16.5± 3.7	17± 1.7	17.5± 3.4	16± 1.4
Fertilized egg diameter (µm)	750 ± 34	750 ± 27	790 ± 32	770 ± 22
Oil drop diameter (µm)	230 ± 17	230 ± 14	240 ± 13	236 ± 16
Larvae length at 1-day old (mm)	1.79 ± 0.4	1.84 ± 0.3	1.76 ± 0.2	1.75 ± 0.2
Larvae length at 2-day old (mm)	2.43 ± 0.3	2.38 ± 0.6	2.36 ± 0.1	2.38 ± 0.2
Larvae length at 3-day old (mm)	2.46 ± 0.2	2.45 ± 0.5	2.44 ± 0.1	2.45 ± 0.4

Figure 3a, 3b, 3c and 3d: Histological stages of sand bass ovaries fully developed at the same stage of the groups.

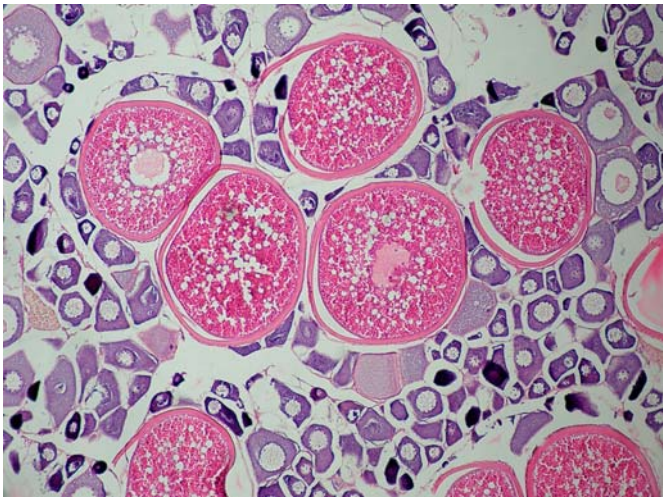

Figure 3a: The ovary section of 5 DOM group.

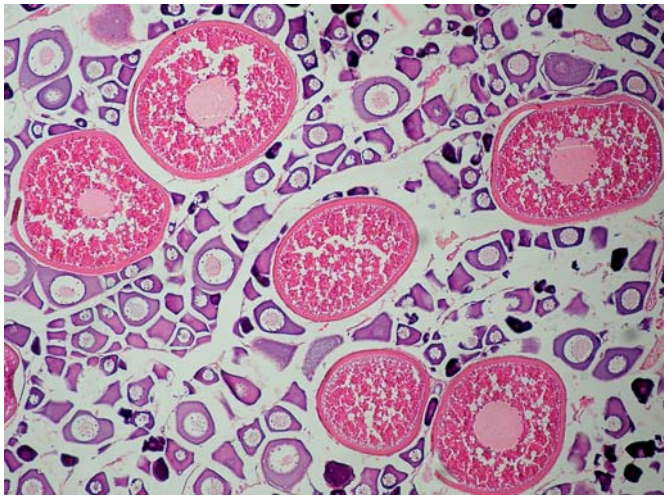

Figure 3b: The ovary section of 10 DOM group.

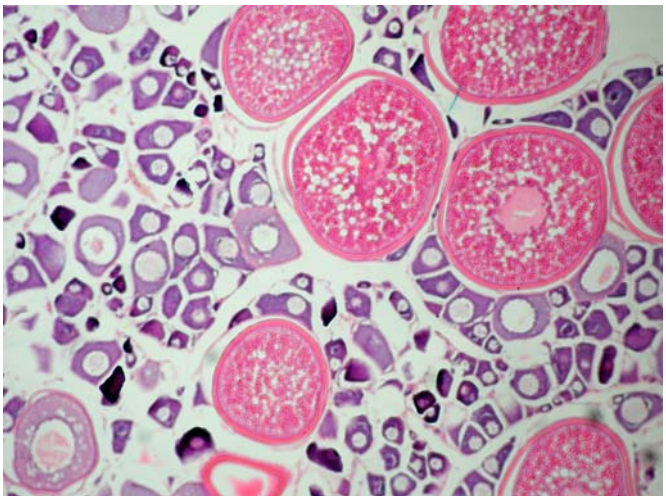

Figure 3c: The ovary section of 15 DOM group.

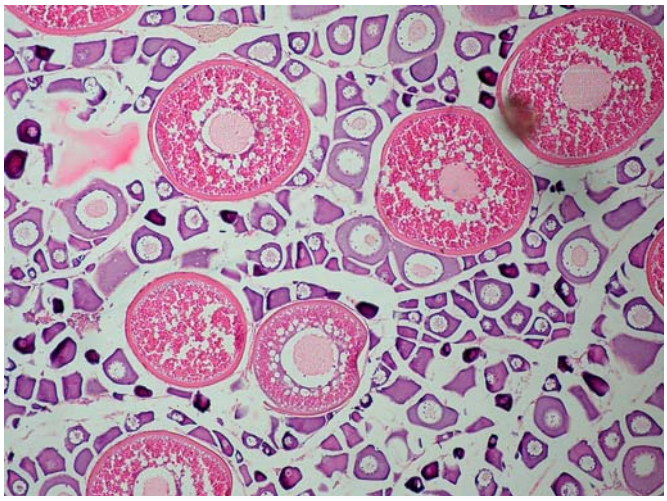

Figure 3d: The ovary section of the control group.

were assessed using one-way ANOVA. Treatment means were compared by least significant difference (LSD) at 95 % confident level. All computations were performed with SPSS 12.0 and excel software program. Values were expressed as mean ± standard deviation (SD).

Results

The present study indicated no significant differences of final oocyte maturation and spawning incidences between the groups treated with different DOM concentration during the breeding season from March to August. The first spawning was observed on April 8, 2007 for all groups after inducing with freshwater and water exchange. During the period from March to August, no significant differences about the number of ovulated females and males between the groups were observed. The inspection for ratio of ovulated females was taken every two weeks and the mean values were 42.34%, 66.28%, 39.45 % and 49.37% for 5 mg DOM, 10 mg DOM, 15 mg DOM and the control (fed no DOM) group, respectively (n = 36). The males were also checked for sperm at the same time as females and found that around 92% males (n= 28) in all groups reached final maturation and released sperm. Table 1 showed the average weight of the females used in this study was from 291.7 gram to 325.2 gram and the length ranged from 24.6 to 27.2 cm. No significant differences of batch fecundity, relative fecundity and egg diameter were found between the groups (Table 1). Table 2 showed egg and larval quality when eggs were incubated at temperature of 30°C. The mean values were derived from 15 spawns during the breeding season.

Discussion

The present study revealed that the sand bass spawned equally well whether they were fed DOM or not during the breeding season. No significant differences of FOMO, egg and larval quality among the groups were found. These might imply the evidence of lacking dopaminergic inhibition of GtH release in sand bass, a marine finfish species. In a study on Atlantic croaker (*Micropogonias undulatus*), Copeland & Thomas (1989) found evidence for lack of dopaminergic inhibition in this species. Maturation of the egg is a long process that involves complex physiological and biochemical changes. One important step, vitellogenesis, is a process in which yolk proteins are produced in the liver, transported to the ovary, and stored in the egg, resulting in tremendous egg enlargement (Barannikova *et al.*, 2002). The first spawning was observed at the same time in all groups after exchanging water implies

that the fish in all groups were at the same stage of gonadal development like at the end of vitellogenesis, for instances. Although these processes are completely regulated by hormones, but the results indicated the role of DA in blocking dopamine and stimulating GtH release in sand bass was not clear. The questions needed to discuss here is that whether the methods of mixing DOM with trash fish applied in this study were reliable and fish could take up the the DOM equally. On the other hand, we questioned whether DOM could be absorbed and transported to the blood stream, and then acted on the hypothalamo-pituitary portal system. Further study needed to be conducted to investigate this mechanism. For more understanding the role of the DOM in this work, measuring of follicle stimulating hormones (FSH) and luteinizing hormone (LH), or any steroid hormones involved in the reproductive process is recommended.

Hormones play a critical role in the reproductive process in teleost fish. They are chemical messengers released into the blood by specific tissues and travel through the bloodstream to other tissues, which respond in a variety of ways. One response is to release another hormone (Bernadette Vidal *et al.*, 2004). The primary tissues involved in this hormonal cascade are the hypothalamus, pituitary gland and gonads. GtH secretion is regulated by a dual hypothalamic control of GnRH and dopamine, which acts as a gonadotropin release inhibitory factor (GRIF) (Barannikova *et al.*, 2002). FOMO in fish is normally initiated by a surge of GtH II (Finn-Arne Weltzien *et al.*, 2005). In this study we investigated the potential of using DOM via oral administration and practically aimed to reduce the injections of hormone, which could stress the fish breeders. Among the most significant advancements in the field of aquaculture during recent decades is the development of techniques to induce reproduction in fish. Feeding DOM in sand bass is the first trial in Vietnam in an attempt to improving the seed production techniques. In an assumption, if DOM via oral administration could enhance spawning in sand bass, this technique might has allowed farmers to advance or extend the spawning season, use fewer breeders and obtain a desired fry production. Consequently, the farmers profitably breed, raise and manipulate the timing of reproduction to suit production cycles.

Hatching rates were not very high and ranged from 38 % to 49 %, lower than those compared with other studies (Nguyen Trong Nho *et al.*, 2003, Syamsul Akbar *et al.*, 2005). This might explain the observed differences were caused by poor male quality and /or water environmental parameters with parasites, which could negatively cause the embryonic development. In the present study, the male size was quite small at around 100 gram each, which gave a small volume of semen. Other reason could also affect the fertilization rate was the ratio between males and females. The learning lesson could be withdrawn from this study was the ratio should be 2 males to 1 female to ensure sufficient sperm for fertilisation if the body size of male was much smaller than female. The sand bass can be easily induced to spawn with water exchange or adding water to stimulate the tide rise. In a study in Thai Carp (*Puntius gonionotus* Bleeker), DOM is the DA has shown a better effect than other DA such as Sulpiride (SUL) and Metoclopramide (MET), which can also stimulate the secretion of GtH II and induce ovulation in fish (Naruepon Sukumasavin *et al.*, 2000). In fish, FOMO is controlled by the maturation inducing steroid (MIS) 17,20-dihydroxy-4-pregnen-3-one (17,20-P) (Nguyen Tuong Anh, 1998). This steroid may also be involved in FOMO in sand bass. Ovulation is often mediated by prostaglandins (PGs) (Barannikova *et al.*, 2001). Therefore, the point raised to discuss are whether MIS and PGs are regulated by the pituitary hormones in sand bass orally treated with DOM during the breeding season. The observation from this study, on the other hand, indicated quality of sand bass larvae was not differences between the groups. This would imply that DOM might not have long-term effect on egg and larval quality in sand bass. Further study might need to be conducted in older larvae and in juvenile stage of sand bass to conclude this assumption.

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