Changes in Serum Concentration of Dopamine, 17β-Estradiol and Progesterone During Reproductive Cycle of Günther's Walking Catfish (*Clarias macrocephalus*)

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ABSTRACT

Seed production of walking catfish (*Clarias macrocephalus*) in Thailand has been declining due to the poor reproductive performance of the female walking catfish. One possible problem might come from the failure of reproductive hormones responsible for ovarian maturation of broodstock. Therefore, this study aimed to investigate the normal patterns of related reproductive hormones coupling with ovarian development of female walking catfish for one full spawning cycle. Blood of female catfish broodstock was collected for hormone analysis by HPLC and RIA. Results showed that at the start of the reproductive cycle (February to April), high DA levels (114.54 ± 28.57 µg/ml) were concomitant with P_4 levels (1.84 ± 0.36 ng/ml). P_4 gradually decreased when entering the spawning period (June to August) until it hits its lowest at 0.78 ± 0.26 ng/ml in August ($p<0.05$), which gave way to an increase of E_2 levels (0.68 ± 0.42 ng/ml). After the spawning season (October to December), DA and E_2 levels in the fish blood declined slightly, while P_4 increased gradually for the next breeding season. The results also showed that the DA and P_4 in female walking catfish were important at the initiation of the pre-spawning season whereas E_2 was crucial for vitellogenesis.

Keywords: dopamine, 17β-estradiol, progesterone, reproductive cycle, Female Günther's walking catfish

INTRODUCTION

A study on the annual reproductive cycle of serum sex steroid hormones and dopamine (DA) of fish is crucial in understanding the endocrine control of reproduction in teleosts (Adebiyi *et al*., 2013). It is well known that, in teleosts, vitellogenesis and final oocyte maturation are regulated by gonadotropins via steroids secreted by the follicular cells surrounding the oocyte. Of these steroids, 17β-estradiol (E_2) stimulates in turn the hepatic synthesis and secretion of vitellogenin which is accumulated in the oocytes. Progesterone (P_4) induces oocyte maturation (Lee and Yang, 2002). Correlations

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between changes in plasma levels of gonadal steroids and oocyte development have been well documented in a number of freshwater species (Adebiyi *et al*., 2013) and marine species (Lee and Yang, 2002). There is no information presently available for the concentration of dopamine (DA) during the annual reproductive cycle of fish. The DA participates in an inhibitory role in the neuroendocrine regulation of the last steps of gametogenesis. In addition, DA may also play an inhibitory role during the early steps of gametogenesis in some teleost species (Dufour *et al*., 2010).

The Günther's Walking catfish (*Clarias macrocephalus*) is a fish species widely distributed from India to Southeast Asia. At present, farmed walking catfish production in Thailand has been declining due to poor reproductive performance (Ingthamjitr, 1997). This study aimed to investigate the normal pattern of related reproductive hormones coupling with ovarian development of female walking catfish in one spawning cycle.

MATERIALS AND METHODS

One year old female walking catfish (MBW = 267.5 ± 72.4 g, total number = 800) used in this study have been reared in net cages in earthen ponds. Female fish (n=20) were collected bimonthly from February to December 2012. Blood samples of fish were collected from the caudal vein. Serum was separated by centrifugation at 3,000 g for 15 min and stored in -80^{o}C until analysis. The ovaries were taken out and weighed, and GSI was calculated. Ovaries were fixed in Bouin's solution, embedded in paraffin after dehydration and infiltration, sectioned at 5-10 mm and stained with hematoxylin and eosin for histological examination (Meunpol, 1988).

Serum levels of E_2 and P_4 were measured by radioimmunoassay. Steroids (E_2 and P_4) were extracted from the serum by double ether extraction. Radio-immuno-assay of plasma steroids followed the method of Kamonpatana *et al*. (1979). Between and within assay coefficients of variation (CV) for E_2 were 8.55% and 8.07%, respectively. The CVs for P_4 were 7.83% and 7.11%, respectively. Serum levels of dopamine were measured by a high performance liquid chromatography-UV detection method, which followed the method of Muzzi *et al*., (2008). A ready-to-use prepacked supelcosil LC-18 column was utilized for the analysis.

Statistical analysis of data was conducted with analysis of variance (ANOVA) followed by Duncan's multiple range test. The relationship between sex steroids hormones was analyzed with Pearson's correlation analysis. The level of significance selected was $p < 0.05$.

RESULTS

Changes in GSI of cultured female walking catfish during the annual reproductive cycle are shown in Figure 1 and Table 1. GSI values increased gradually at pre-spawning (February to April), and were highest during the spawning season (June to August). GSI values dropped gradually at post-spawning stage (October to December). Oocyte

Table 1. The values of gonadosomatic index, ovarian stage (%) and hormone leves (DA, E_2 and P_4) present as mean±SD (n=20) in female Günther's walking catfish (*Clarias macrocephalus*)

	February	April	June	August	October	December
Gonadosomatic index (GSI)	2.6±1.9^{c}	5.2±3.7^{bc}	10.5±2.6^{a}	9.0±2.6^{a}	7.1±4.5^{ab}	3.9±3.5^{bc}
Ovarian stage (%)						
Previtellogenic oocyte (%)	44.8±13.1^{a}	31.8±15.4^{a}	12.3±7.0^{b}	14.0±7.4^{b}	37.8±15.6^{a}	43.9±13.6^{a}
Vitellogenic oocyte (%)	24.6±14.2^{a}	19.9±7.6^{a}	21.8±5.1^{a}	19.0±8.1^{a}	16.9±5.8^{a}	15.4±10.9^{a}
Postvitellogenic oocyte (%)	30.6±17.8^{b}	48.2±21.0^{ab}	65.9±8.2^{a}	66.6±9.6^{a}	34.4±20.3^{b}	32.0±24.5^{b}
Atretic oocyte (%)	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	10.9±11.9^{a}	8.7±8.3^{a}
Hormone levels						
Dopamine (μg/ml)	114.5±28.6^{a}	105.0±17.3^{a}	57.6±24.8^{b}	49.1±17.4^{b}	24.3±13.1^{c}	18.4±9.3^{c}
17β-estradiol (E_2) (ng/ml)	0.5±0.3^{ab}	0.4±0.2^{ab}	0.3±0.2^{b}	0.7±0.4^{a}	0.4±0.3^{ab}	0.6±0.5^{ab}
Progesterone (P_4) (ng/ml)	1.8±0.4^{a}	1.5±0.2^{ab}	1.6±0.4^{ab}	0.8±0.3^{d}	0.9±0.4^{cd}	1.3±0.7^{bc}

* Superscript letters indicate significant differences on the same rows (P<0.05)

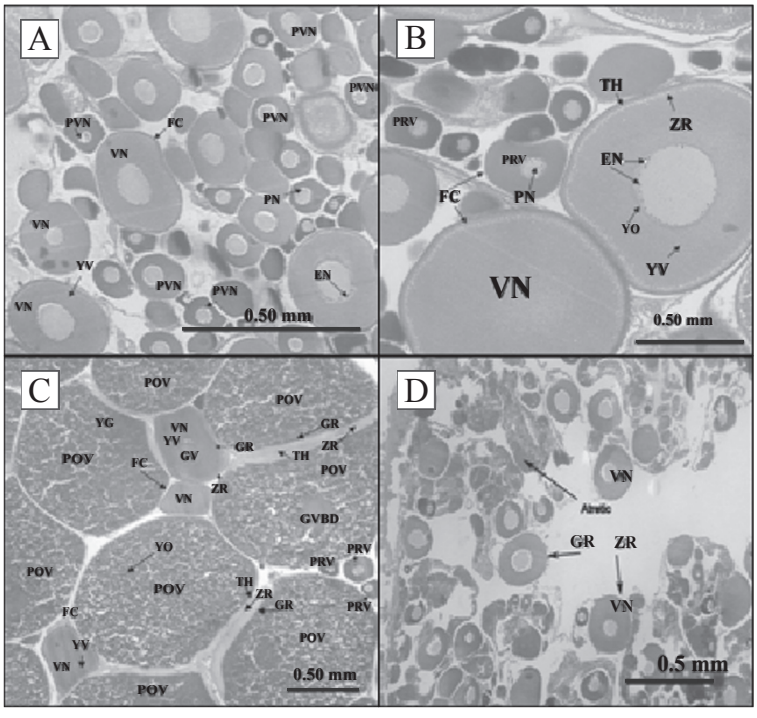

Figure 1. Oocyte developmental stages. A–D: Ovaries of Günther's Walking catfish (*Clarias macrocephalus*). H&E stain, bar equals 0.50 mm. A: pre-vitellogenic oocytes; B: vitellogenic oocytes; C: post-vitellogenic oocytes; D: atretic stage. (PRV: pre-vitellogenic oocyte; VN: vitellogenic oocyte; EN: euvitelline nucleoli; FC: follicular cell; PN: provitelline nucleoli; YV: yolk vesicles; POV: post-vitellogenic oocyte; GV: germinal vesicle; GVBD: germinal vesicle breakdown; ZR: zona radiate; GR: granulosa)

development in the walking catfish is divided into 4 stages according to the general classification of the teleost gonad (Meunpol, 1988), namely: pre-vitellogenic oocyte, vitellogenic oocyte, post-vitellogenic oocyte, and atretic oocyte (Figure 1). The GSI values had a highly positive correlation with post-vitellogenic stage (Pearson's correlation, $r = 0.761$, $p = 0.000$). It also had a highly negative correlation with pre-vitellogenic oocyte ($r = -0.765$, $p = 0.000$) and atretic oocyte ($r = -0.555$, $p = 0.000$). Vitellogenic oocyte was not related to the GSI values ($r = -0.178$, $p = 0.060$).

Table 1 shows the percentages of oocytes. The maximum percentage of post-vitellogenic oocyte was found during the spawning season, after which it decreased during the post-spawning season. The percentage of pre-vitellogenic oocytes decreased during the spawning season, then increased from the post-spawning season until the end of the pre-spawning season (February to April). This was followed by vitellogenic oocytes, with their ratios remaining constant throughout the year. The percentage of atretic stage was found at post-spawning (October to December).

Female walking catfish also showed seasonal variation in serum DA, E_2 and P_4 levels (Table 1, Figure 2). During the beginning of the reproductive cycle (February to April), high levels of DA (114.5 ± 28.6 µg/ml) were related to P_4 levels (1.8 ± 0.4

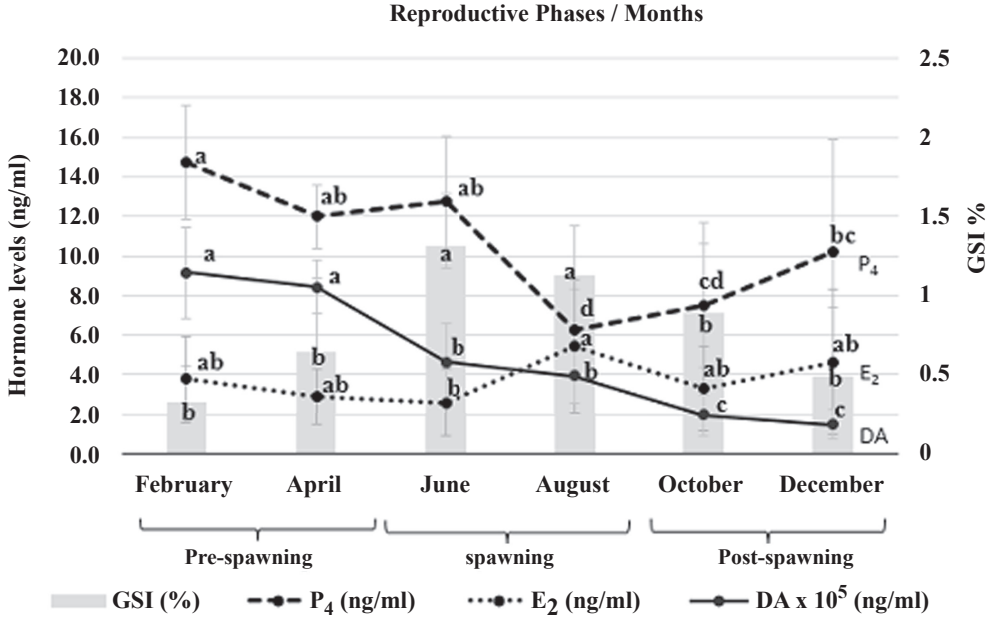

Figure 2. Bimonthly variations in serum levels of 17β-estradiol (E_2), progesterone (P_4), dopamine (DA) and in the gonadosomatic index (GSI, %), during annual reproductive phases in the female Günther's walking catfish (Clarias macrocephalus). Values represent Mean ± SD and letters indicate significant differences ($p < 0.05$, n=20).

ng/ml) ($p<0.05$) and low levels of E_2 (0.5 ± 0.3 ng/ml - 0.4 ± 0.2 ng/ml). During the spawning period (June to August), serum E_2 levels were lowest at 0.3 ± 0.2 ng/ml ($p<0.05$) in June, and began to increase, reaching the peak at 0.7 ± 0.4 ng/ml, while the P_4 levels decreased and were lowest at 0.9 ± 0.4 ng/ml in August ($p<0.05$). In the meantime, serum DA was decreased slightly. During the post-spawning period (October to December), DA was lowest at 18.4 ± 9.3 µg/ml in December while E_2 decreased slightly and P_4 increased gradually.

The results of present study revealed that changes in serum levels of E_2 were closely correlated to ovarian development and GSI increase ($r = 0.191$, $p = 0.043$). The GSI values had a negative significant correlation with the DA ($r = -0.227$, $p = 0.016$). There was no significant correlation between E_2 in all females and DA ($r = -0.132$, $p = 0.164$). There was a negative correlation between P_4 in all females and E_2 ($r = -0.198$, $p = 0.036$), and a positive correlation between P_4 in all females and the change in serum DA levels ($r = 0.467$, $p=0.000$) (Figure 2).

DISCUSSION

The ovary of female walking catfish was small at pre-spawning (February to April), predominated by pre-vitellogenic oocytes with concomitant low E_2. The data collected during the investigation showed that there were highly positive correlations between the serum levels of DA and P_4 during the pre-spawning season, thus implying that P_4 may be related to the regulation of DA, and both hormones may also be significantly involved in the early stage oocyte development (Atteke *et al*., 2003). P_4 involves the mechanic pathway of E_2 production for vitellogenesis during the spawning phase and no clear role by itself has been reported (Atteke *et al*., 2003).

During the spawning period (June to August), serum E_2 levels were high, probably coinciding with the recruitment of oocytes into yolk accumulation (Pham *et al*., 2010). Changes in serum E_2 levels generally were correlated with oocyte development in the ovary and increases in GSI. The up-regulation by E_2 of DA inhibitory tone during vitellogenesis thus supports the adult-specific control at the last steps of gametogenesis (Dufour *et al*., 2010) and maintain a dopamine inhibitory tone of LH (Vacher *et al*., 2002). In walking catfish, the post-spawning in October to December corresponds to the decreasing level of post-vitellogenic oocytes, GSI, E_2 and DA. The DA should be lowest at the end of gametogenesis, under the control of environmental cues (photoperiod and temperature) (Dufour *et al*., 2010).

CONCLUSION

In the beginning of the spawning season, P_4 displayed higher values than E_2. During the spawning season, E_2 became the dominant steroid and the actual hormone in the circulatory system of the walking catfish. Finally, both steroid hormones were maintained at equally high level. Dopamine showed a peak in the pre-spawning season and reduced gradually in the spawning season until it reached its lowest during the

post-spawning season. In the pre-spawning season, the highest levels of P_4 and DA, and the lowest level of E_2, were found. The relationship of P_4 and DA was directly related to a high percentage of previtellogenic oocyte. In the spawning season, high serum E_2 levels were found, which correlated with direct postvitellogenic oocyte and involved in the later stages of oocyte development. During this time, P_4 was at its lowest. Later on, during the spawning season, E_2 became the dominant steroid hormone, in contrast with P_4. In the post-pawning season, high P_4 levels were found whereas E_2 and dopamine levels were. Thus, P_4 may be involved in early stage oocyte development. This study provides information to better understand the hormonal regulation in reproductive processes in female walking catfish.

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