Annual Growth and Reproductive Performance in an F_2 Catfish Hybrid

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***Abstract*—Association among growth performance, sex hormone levels, and gonad histology of fifty-four one year old hybrid F_2 (*Pangasianodon gigas* x *P. hypophthalmus*) were mekong giant catfish and striped catfish hybrids, observations were made during the following periods pre-spawning (March), spawning (June), and post-spawning (October). The overall weight gains of female (174.5±41.3 to 431.8±41.3 g) and male fish (170.0±51.7 to 537.1±51.7 g) were similar. The, average daily gain (ADG of males collected in October (3.24±0.3 g/day) was higher than in June and March. Similarly, the specific growth rate (SGR) of female (0.18±0.02) and male fish (0.25±0.03) were higher in October than in June and March. The highest testosterone values in male fish were observed in June. Gonad histology revealed that spermatozoa were always present in the testes. However, no mature ovaries were observed. More collecting data are needed for further study. However, initiation phase showed that the results indicate that male hybrids mature more quickly than female hybrids the first two years of life. This study showed there was potential for future selective breeding programs with mekong giant catfish and striped catfish hybrids.**

***Index Terms*—Growth performance, sex hormone level, gonad histology, hybrid catfish F_2**

I. Introduction

Freshwater catfish in the Family Pangasiidae are an important source of protein for local consumers in South-east Asian countries and also have commercial potential “Ref. [1]”. Artificial breeding programs, producing a second generation (F_2, the Blabuk Maejo 75) of the mekong giant catfish (*Pangasianodon gigas*), in earthen ponds have thus far been successful “Ref. [2]”. The establishment of these breeding programs has ensured the survival of the mekong giant catfish, preventing its extinction. Be that as it may, they still remain on the red list of critically endangered species “Ref. [3]”. This may be a result of the limited amount of registered data that is available on the annual catch of the mekong giant catfish in the Mekong River at Chiang Kong District in Chiang Rai Province of Thailand, during the period of 1986-2007. There was a maximum number of 65 fish caught in 1990. While in later years, this number has declined to just one or two (in 2000 and 2003-2006), and to zero (in 2001, 2002 and 2007) “Ref. [2]”. Food that is required to sustain the fish during the process of aquaculture is not met from its natural environment alone “Ref. [4]”. Feed need to be introduced, in order to further its development and for efficient cultivation. Hybridization involves producing a progeny from the parents of a different line, strain, and or species “Ref. [5]”. Hybrid strains offer increased growth rates and yields, as well as greater disease resistance, in comparison to the pure line species “Ref. [6], [7]”. In terms of reproduction, *P. gigas* and *Pangasianodon hypophthalmus* (striped catfish) showed signs of a seasonal gonadal maturation, typically mating during the rainy season (May-August) “Ref. [8]”. Meng-umphan and Saengkrachang “Ref. [9]” reported that *P. gigas* that are raised in captivity, whether it be in rivers, lakes, or ponds, required at least 10-15 years for maturation. While *P. hypophthalmus*, the species that was raised in captivity in earthen ponds, needed only least three years “Ref. [10]”. Recently, Panase *et al*. “Ref. [11]” showed that the generation one (F_1) hybrid catfish (*P. gigas*, male x *P. hypophthalmus*, female) were matured and ready for propagation earlier than both *P. gigas* and *P. hypophthalmus*. However, there has been a lack of accumulated information on the growth and reproductive capabilities of the hybrid F_2 catfish. The aim of the present study was to investigate the growth performance, sex hormone levels, and the gonad histology of the Hybrids F_2 during its pre-spawning, spawning, and post-spawning periods.

II. Meterial and Methods

Fish samples: three replications of three treatments in completely randomized design (CRD) were designed in the experiments and was conducted from January to October of 2013. A total of fifty-four Generation two (F_2) hybrids (27 male and 27 female, starting at one year old) of *P. gigas* (male) crossbred with *P. hypophthalmus* (female) were reared in the same earthen pond (300 m^2) at the Faculty of Fisheries Technology, Maejo University, Chiang Mai. The average initial body weight of fish was 494.3±21.2 g, a pectoral fin fish of male and female were tagged with alcian blue (Sigma, USA). The fish were fed daily on pellet feed containing 30% protein at 3% of their body weight.

Growth performance: the body weight of all the fish were recorded every three months; from January 2013 to the end of October 2013. Growth performance was described using four commonly used parameters: weight

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gain (WG), average daily gain (ADG), specific growth rate (SGR), and feed conversion rate (FCR). The following formulae were used to calculate these parameters:

Weight gain (WG) = final weight-initial weight
Average daily gain (ADG) = final weight-initial weight/ days
Specific growth rate (%SGR) = (ln final weight-ln initial weight) x 100/days
Feed conversion ratio (FCR) = total feed (g)/weight gain (g)

Gonadosomatic index (GSI): nine male and nine female fish were randomly sampled every three months (from each spawning season). The fish were anaesthetized with clove oil (0.1 ml/l), and then sacrificed. The body weight and gonad weight of all the fish were measured using a digital scale with a 0.01 decimal sensitivity. The GSI of the ovaries and testis were calculated using the following equation from Nikolsky "Ref. [12]":

Gonadosomatic idex (%GSI) = gonad weight x 100/body weight

Testosterone and estradiol analysis: Blood samples were collected from the caudal vein of each fish in March (1.3 years old), June (1.6 years old), and October (1.9 years old). One-milliliter blood samples were centrifuged at 10,000 rpm for 5 minutes, and the serum was stored at -80°C, after which, 17β-estradiol (E_2) and testosterone (T) levels were measured by an electro-chemiluminescence immunoassay analyzer (Elecsys 2010, Roche, Germany).

Gonad histology: ovary or testis samples were fixed in a solution of 10% formalin. Fixed ovaries were then dehydrated with a graded series of ethanol solutions, and embedded in paraffin wax. Sections 4 µm thick were stained with haematoxylin and eosin. The middle parts of the samples were examined under a light microscope, at magnification levels of 100x and 400x. Oocyte developmental stages were classified according to Coward and Bromage "Ref. [13]".

Data and statistical analysis: estimates of growth performance, %GSI, 17β-estradiol (E_2), and testosterone (T) levels, were analyzed using analysis of Two-way ANOVA. Pearson product moment correlations were estimated to measure the strength of association between pairs of variables.

III. Results and Discussion

Growth performance: The growth performance of the F_2 catfish that were reared in earthen pond conditions, during the period from January 2013 to October 2013, are presented in Table I. Significant increases in values were observed in the weight gains and of the male population. These results showed that the values in October were higher than in March and June (*P<0.05*). Similarly, hybrid F_2 males also increased, particularly in weight gain, ADG, and % SGR, but were not difference in June. Moreover, SGR of the hybrid F_2 increased in the collected data. This contrasts to the results found by Kerby *et al*. "Ref. [14]," wherein it was reported that SGR decreased with increasing in age.

In terms of ADG, we found that the ADG of the male specimens in October was higher than in June and March (Table I). A similar observation was reported by Kadri *et al*. "Ref. [15]". They reported that with many species, feed intake could also be reduced or inhibited completely prior to and/or during the spawning period. Brown et al. "Ref. [16]" reported that the growth rate of the hybrid catfish (*I. punctatus* x *Ictalurus furcatus*) ranged from 1.6 to 2.2 g/fish/day. There were no significant differences recorded during all months for both male and female specimens (Table I). The average FCR for hybrid catfish (*I. punctatus* x *Ictalurus furcatus*) was 1.36. This suggests that catfish can be grown to commercial size (1.5 kg) with an efficient FCR of less than 1.8 "Ref. [16]".

TABLE I. Growth and Reproductive Performance Characteristics of Female and Male Hybrid Catfish F2 (*P. Gigas x P. Hypophthalmus*) from Pre-spawning (Jan-Mar), Spawning (Mar-Jun), and Post-Spawning (Jun-Oct).

parameter	Female		
	Jan-Mar	Mar-Jun	Jun-Oct
WG	174.54 ± 41.3^{a}	387.27 ± 41.31^{bc}	431.81 ± 41.31^{c}
ADG	0.86 ± 0.26^{a}	1.92 ± 0.26^{bc}	2.48 ± 0.26^{c}
% SGR	0.15 ± 0.02^{a}	0.22 ± 0.02^{ab}	0.18 ± 0.02^{b}
FCR	1.88 ± 0.27^{a}	1.26 ± 0.27^{a}	1.48 ± 0.27^{a}
%GSI	0.17 ± 0.15^{a}	0.22 ± 0.15^{a}	0.16 ± 0.13^{a}
E_2 level	24.56 ± 4.37^{a}	46.27 ± 10.88^{a}	49.05 ± 8.60^{a}
parameter	Male		
	Jan-Mar	Mar-Jun	Jun-Oct
WG	170.0 ± 51.78^{a}	288.57 ± 51.78^{a}	537.14 ± 51.78^{b}
ADG	0.84 ± 0.32^{a}	1.43 ± 0.32^{a}	3.24 ± 0.32^{b}
% SGR	0.11 ± 0.03^{a}	0.14 ± 0.03^{ab}	0.25 ± 0.03^{b}
FCR	2.07 ± 0.27^{a}	1.71 ± 0.27^{a}	1.05 ± 0.27^{a}
%GSI	0.134 ± 0.19^{a}	3.07 ± 0.19^{b}	0.231 ± 0.17^{a}
T level	NF	1.33 ± 0.35	NF

WG = weight gain, ADG = average daily gain, % SGR = specific growth rate, FCR = feed conversion rate, and %GSI = gonadosomatic index, E_2 = 17β-estradiol, T = testosterone, NF = not found. ^{a,b,c} = difference is significant at the 0.05 level.

Gonad maturity: in this study the GSI of the hybrid F_2 females during the sampling months were low ranging from 0.16% to 0.22%, with no statistically significant difference between times. On the other hand, GSI levels for the males were higher in June than other two times (Table I). Our results show that the hybrid catfish F_2 males matured earlier than their female counterparts. Meng-umphan *et al*. "Ref. [8]" reported that the GSI of the female *P. gigas* cultured in earthen ponds were 0.36±0.05% if immature and 7.91±2.62% if mature. Whereas, the GSI levels for the male fish were 0.05±0.03% if immature and 2.21% if mature. Other catfish, such as the *Hemibagrus nemurus*, had GSI levels ranging from 1.14±0.02% to 7.06±1.40%, with high GSI recordings in May, August and November "Ref. [17]". On the other hand, South American female catfish at 3 years old (*Pseudoplatystoma* sp.) were found to have a GSI of only 0.42±0.14% in March of 2003, which indicated that this fish had not yet matured "Ref. [18]". From our study, due to a lack of presence of eggs, we concluded that no effective spawning had taken place within the female population. Despite this, we found two hybrid F_2 males that could release sperm at about 9.00 and 10.00 ml levels during the spawning period (June).

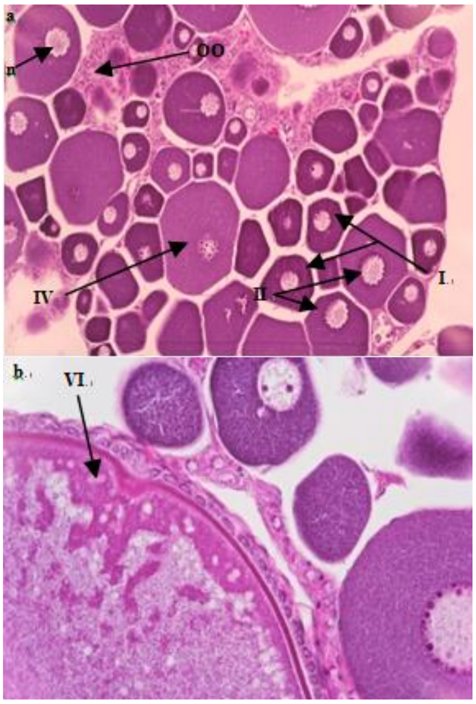

Figure 1. The cross-section of the ovary of the hybrid F_2 showing more than two stages of oocyte development: a) Immature ovary, b) Mature ovary, *n* (nucleus), *oo* (oogonium), *I* (chromatin nucleolar oocyte), *II* (early perinucleolar oocyte), *IV* (cortical alveolar oocyte), *VI* (mature oocyte).

A histological examination of all of the hybrid F_2 females revealed that the following ovary development stages: oogonium, chromatin nucleolar oocyte, early perinucleolar oocyte, and cortical alveolar oocyte. However, in October, only one cell of one hybrids F_2 female was found to possess a mature oocyte (Fig. 1). Most stages of oocyte were found in the previtellogenesis stage, but none were observed in the final oocyte maturation stage. This result indicates that the female hybrid F_2 catfish were immature in terms of developing an oocyte. A similar pattern was observed in female *P. gigas*, whereby oogonia and oocyte were recorded during all seasons (February-November) "Ref. [8]". In the *P. hypophthalmus* specimens, the oocyte development stage appeared to take place more often in June and September instead of in February and November "Ref. [10]". The gonads of female hybrid F_2 catfish at 2 years old may be like those of other catfish (Asian catfish (*Clarias macrocephalus*) and Japanese catfish (*Silurus asotus*)), that were raised in captivity, revealed a synchronous pattern of oocyte development "Ref. [19], [20]". However, female hybrid F_2 catfish at two years old were not mature. The reasons for this remain unclear, and more time is required to collect additional data.

In terms of the male hybrid F_2 catfish histology, we found that spermatogonia, spermatocytes, spermatids, and spermatozoa, were present during all months (March, June and October) (Fig. 2). Moreover, our observations founded that in June and October, there were higher recorded levels of spermatozoa than in March. The development of spermatocytes was also found in the eight-year old *P. gigas* which was reared in earthen ponds in during May (spawning season) "Ref. [21]". Our results indicated that the male hybrid F_2 catfish underwent continuous spermatogenesis at maturation. Similar results were obtained for the following species: *Cyprinus carpio* "Ref. [22]," *Tor tambroides* "Ref. [23]," and *H. nemurus* "Ref. [17]".

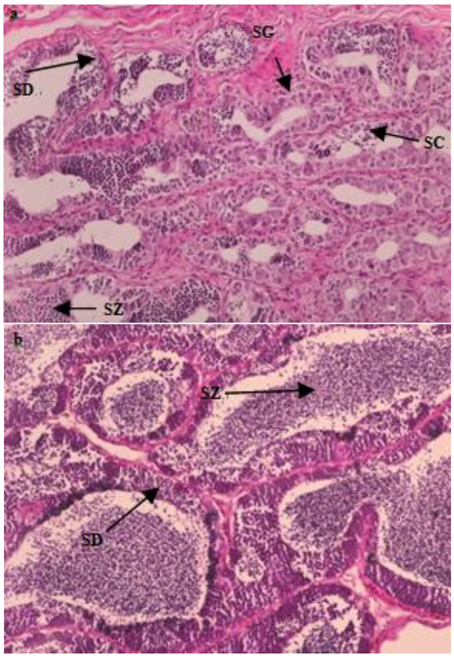

Figure 2. A cross-section of the testis of hybrid F2 consisting of: **a)** Immature testis, **b)** mature testis *SG* (spermatogonium), *SC* (spermatocyte), *SD* (spermatid), *SZ* (spermatozoa).

17β-estradiol and testosterone levels: In females, 17β-estradiol (E_2) concentrations were not found to be significantly different ($P>0.05$) in March, June, and October (Table I). The hormone regulation of female fish is determined by 17β-estradiol (E_2), but is formed in male vertebrates, as well "Ref. [24]-[26]". Our results showed that 17β-estradiol (E_2) concentrations in female hybrid F_2 catfish were not found to be significantly different ($P>0.05$) in March, June and October, which were recorded at approximately 24.56±4.37, 46.27±10.88 and 49.05±8.60 pg/ml, respectively. This result indicates that the two-year old female hybrid F_2 catfish had not yet matured and therefore did not undergo vitellogenesis. However, a rise in 17β-estradiol only appears in mature oocytes, as this initiates vitellogenesis "Ref. [27]". The nine-year old female *P. gigas* specimens that were cultured in earthen ponds, showed the highest levels of 17β-estradiol in May, at 47.28±37.28 pg/ml, and these specimens may not yet have even reached sexual maturation "Ref. [8]". Other catfish species, such as *Rhamdia quelen* and *H. nemurus*, were observed to possess the highest 17β-estradiol levels in November "Ref. [17], [27]".

Based on the knowledge of sex steroid hormone levels, testosterone is a male specific androgen and plays an important role in controlling fish spermatogenesis "Ref. [28]-[34]," although testosterone and 11-ketotestosterone are present in the blood plasma of female fish "Ref. [35], [36]". Testosterone concentration levels of the male hybrid F_2 catfish were significantly higher in June (1.33 ± 0.35 ng/ml) than in March and October ($P<0.05$); significant testosterone levels were not found (<0.025 ng/ml) in March and October (Table I); serum sex steroid showed the same seasonal changes as the mean GSI values (Table I). This indicates that of testosterone levels were highest during spawning season, which accorded with the period of active spermatogenesis. Similar, observation was reported in red-spotted grouper (*Epinephelus akaara*) by Li *et al*. "Ref. [37]".

On the other hand, the testosterone levels in male *P. gigas* fish were highest in May (0.06±0.04 ng/ml) "Ref. [8]". Moreover, in male *H. nemurus* catfish, several peaks of testosterone levels were observed in October, November, and June, while those of 11-ketoestosterone were observed in October, February, and June "Ref. [17]". *Tor tambroides*, by Ismail *et al*. "Ref. [23]," also observed a similar pattern in the levels of testosterone and 11-ketoestosterone.

TABLE II. CORRELATION COEFFICIENTS (R) AMONG THE REPRODUCTIVE PARAMETERS OF HYBRID CATFISH F_2 FEMALE AND MALE SPECIMENS (*P. GIGAS X P. HYPOPHTHALMUS*) DURING THE PERIOD OF JANUARY-OCTOBER, 2013.

Female	BW	OW	%GSI	E_2 level
BW	1.000			
OW	0.219	1.000		
%GSI	-0.171	0.887*	1.000	
E_2 level	0.610*	0.604*	0.418	1.000
Male	**BW**	**TW**	**%GSI**	**T level**
BW	1.000			
TW	0.090	1.000		
%GSI	-0.146	0.903*	1.000	
T level	-0.256	0.408	0.627 *	1.000

BW = body weight, OW = ovary weight, TW = testis weight, %GSI = gonadosomatic index, E_2 = 17β-estradiol, T = testosterone, *Significant difference ($P<0.05$)

Correlation analysis: in the hybrid F_2 female specimens, % GSI and 17β-estradiol levels were both positively correlated with ovary weight (Table II). However, only 17β-estradiol showed a positive correlation with body weight; a negative correlation was found between body weight and %GSI. On the other hand, Cornish *et al*. "Ref. [38]" observed that the increase of plasma 17β-estradiol levels in female Tilapia (*Oreochromis mossambicus*), paralleled the increase of its GSI levels. In addition, hybrid F_2 male testis weight and testosterone levels were positively correlated with % GSI. However, a negative correlation was found in % GSI and testosterone levels with body weight (Table II). Similarly to Cornish *et al*. "Ref. [38]," they reported that the increases of plasma 17β-estradiol levels in female Tilapia (*Oreochromis mossambicus*) were parallel with the increases of GSI levels, whereas, we reported a negative correlation between body weight and GSI. In hybrid F_2 male catfish, testis weight and testosterone levels were positively correlated with the GSI. However, a negative correlation was found in the GSI and testosterone levels with body weight (Table II). A relationship between GSI and body weight of, both male and female specimens revealed a negative correlation. According to Mann "Ref. [39]," the GSI was also found not to increase with body weight. However, Mahboob and Sheri "Ref. [40]" reported that the GSI increased with body weight in the species *C. idella*. Additionally, in the species, *Mastacembelus armatus* and *Rutilus rutilus*, a positive relationship between GSI and body weight was also established "Ref. [41], [42]".

IV. CONCLUSION

Hybrid F_2 catfish were studied during pre-spawning (March), spawning (June), and post-spawning (October) periods. Growth parameters showed a high potential trend in weight gain, ADG, SGR, and FCR, for both male and female catfish. The levels of the female's sex steroid hormone, 17β-estradiol, were not found to be significantly different in March, June, and October. However, the testosterone levels of the male catfish were highest in June. Gonad histology revealed that, female hybrid F_2 catfish showed different stages of oocyte development, including for oogonium, chromatin nucleolar oocyte, early perinucleolar oocyte, and cortical alveolar oocyte, in the month of March, June, and October. However, only one mature oocyte was found in October. The male hybrid F_2 catfish histology showed that the spermatogonia, spermatocytes, spermatids, and spermatozoa, were present during all periods. There results indicate that the male hybrid F_2 become sexually mature at a younger age than female. The satisfactory growth rate observed provide further indication of the potential of aquaculture production of the hybrid catfish for commercial purposes, but further and long-term studies on growth and reproductive cycle are encouraged.

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