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**Gonadotropin Induced Synchronous Changes of Morphology and
Gonadal Development in the Japanese Eel *Anguilla japonica***

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Abstract

In a previous study, we had observed synchronous changes of morphology and gonadal development in wild Japanese eel during the silvering process. In this study, we aimed to clarify if gonadotropin is the key hormone responsible for this phenomenon. Yellow eels captured in the Kaoping River were repeatedly injected with human chorionic gonadotropin (HCG), and changes of morphology and gonadal development were examined. After five weekly injection of HCG at a dosage of 0.75 IU/g body weight, eels was classified into well-responsive and poor-responsive groups according to skin coloration. Approximately 50 % of males and 20 % of females were responsive to become silver eels. Mean age, total length, body weight, condition factor, gonadosomatic index (GSI), fin-index (FI), hepatosomatic index (HSI) and ocular index (OI) were significantly higher in well-responsive eels than in poor-responsive eels. The mean digestosomatic index (DSI), in contrast, significantly decreased in well-responsive eels compared to poor ones. OI was positively correlated and DSI was negatively correlated with the GSI in both sexes. These results indicated that well developed eels are more sensitive to HCG treatment, and the skin color, eye size, gonadal development and digestive tract shrinkage were synchronous after HCG injection. The pituitary – gonad axis plays important role on eel silvering.

Key words: Japanese eel; Silvering; Gonadal development; Morphology; Human chorionic gonadotropin

摘要

先前的研究中已證實，野生日本鰻在銀化過程中形態上的改變與生殖腺的發育呈現同步化現象。在本研究中，吾人擬探討促性腺激素是否扮演關鍵的角色。將高屏溪採補到之日本鰻黃鰻每週施打人類絨毛膜促性腺激素（HCG），檢驗樣本在形態上的改變與生殖腺的發育模式。經過五週的施打後（0.75 IU/克體重），根據鰻魚體色的變化將其分為反應良好組與反應不良組。大約有 50 % 的雄鰻與 20 % 的雌鰻反應良好，變態為銀鰻。反應良好組其平均年齡、體長、體重、肥滿度、性腺指數（GSI）、胸鰭指數（FI）、肝指數（HSI）以及眼徑指數（OI）皆顯著高於反應不良組。相反地，消化道指數（DSI）在反應良好組則顯著低於反應不良組。性腺指數與眼徑指數呈現正相關，而與消化道指數呈現負相關。發育程度較佳之鰻魚對促性腺激素之反應較為敏感，而在促性腺激素施打後，日本鰻體色、眼徑指數與消化道指數之變化與性腺之發育呈現同步化現象。結果顯示，腦下垂體-性腺軸在日本鰻銀化過程中扮演重要的角色。

關鍵詞： 日本鰻； 銀化； 性腺發育； 形態； 人類絨毛膜促性腺激素

1 Introduction

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3 The catadromous Japanese eel *Anguilla japonica* has a complex and special life cycle (Tesch,

4 1977). After living in rivers for years, the eel undergoes significant morphological and physiological

5 changes from yellow (non-migratory) to silver (migratory) stages (also known as “silvering”). The

6 morphological modifications include a change in skin color from yellow to silver/bronze (Tesch, 1977;

7 Han et al., 2003a), integumental thickness (Tesch, 1977; Sorensen and Pankhurst, 1988), increased

8 eye size (Pankhurst, 1982a; Rohr et al., 2001; Han et al., 2003a), and changes in the shape of pectoral

9 fins and snout (Tesch, 1977; Rohr et al., 2001). The physiological changes include degeneration of

10 digestive tract (Pankhurst and Sorensen, 1984; Han et al., 2003a), changes of visual pigments

11 (Pankhurst, 1982a), modification in the composition and function of skeletal muscle and bone

12 (Lewander et al., 1974; Pankhurst, 1982b; Ellerby et al., 2001; Yamada et al., 2002), more developed

13 swim bladder (Kleckner, 1980; Yamada et al., 2001), higher density of chloride cells in the gill

14 (Fontaine et al., 1995), higher muscle fat contents (Han et al., 2001), lower non-esterified fatty acids

15 (Cottrill et al., 2001), and more developed gonad (Colombo et al., 1984; Jessop, 1987; Lokman et al.,

16 1998; Lokman and Young, 1998; Han et al., 2003a). The changes in endocrine profiles include the

17 increase of sex steroids (Lokman et al., 1998; Lokman and Young, 1998; Cottrill et al., 2001; Han et

18 al., 2003b, 2003c) and thyroid hormone (Han et al., 2004), and the decrease of growth hormone

19 (Marchelidon et al., 1996), cortisol (Lewander et al., 1974; Lokman et al., 1998) and prolactin (Han et

20 al., 2003d). These modifications are proposed to be a pre-adaptation for marine environment before

21 spawning migration of the silver eels (Pankhurst, 1982a, 1982b; Fontaine et al., 1995; Han et al.,

22 2003a).

23

24 In captivity, eels have immature gonads containing mainly pre-vitellogenic oocytes in females

1 and pre-meiotic spermatogonia in males, which were due to insufficient synthesis and release of
2 gonadotropin (Quérat et al., 1991). However, a fully mature gonad of the Japanese eels can be
3 induced by multiple injections of salmon pituitary homogenates or human chorionic gonadotropin
4 (HCG) (Yamamoto and Yamauchi, 1974; Miura et al., 1991; Ohta et al., 1996; Liao and Chang, 1999
5 2001; Chang et al., 2003, 2004). In a previous study, the gonadal development of the wild Japanese
6 eel was found to be synchronous with the changes of skin color, eye size, and digestive tract before
7 and during silvering process in both sex, and the skin coloration and ocular index were reliable criteria
8 in selecting mature eel brooders for artificial propagation purpose (Han et al., 2003a). The
9 synchronous phenomenon seems to be under endocrine control. In female Australian eel *Anguilla*
10 *australis*, injection of androgen (11-ketotestosterone) resulted in gonadal development and some
11 silvering-related changes, such as chisel-shaped snouts, black pectoral fins with tapered end, thicker
12 dermis and enlarged eyes and livers (Rohr et al., 2001). In female European eel *Anguilla anguilla* and
13 American eel *Anguilla rostrata*, the development of gonads and degeneration of alimentary tract were
14 observed after injection of human chorionic gonadotropin (HCG) (Pankhurst and Sorensen, 1984).
15 Accordingly, these findings inferred that the hypothalamus - pituitary - gonad (HPG) axis might play
16 an important role on the gonadal development accompanied with the morphometric changes during
17 eel silvering. If this is true, the synchronization between gonadal development and morphometric
18 index should also be observed when yellow eels of both sex were induced to mature by HCG
19 injection.

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21 This study aimed to examine the relationship between morphological changes and gonadal
22 development of the Japanese eels after induced maturation by repeated HCG injection. Results were
23 compared with those of wild yellow and silver eels to better understand the silvering pattern of the
24 Japanese eel.

Materials and methods

Eel collection and silvering induction

Yellow Japanese eels were collected from Kaoping River estuary in southwestern Taiwan (120° 50' E and 22° 40' N) in August 2002, and reared in the laboratory for three months using freshwater at ambient temperature and natural photoperiod. A total of 58 randomly selected yellow eels with total length (TL) above 40 cm were used for HCG injection in November 2002, while 16 yellow eels with TL above 40 cm were mock-injected with normal saline as the control group. The eels were injected five times (once per week) with HCG at a dosage of 0.75 IU/g body weight. One week after the last injection, the eels were anesthetized with 0.05 % 2-phenoxyethanol for internal morphometric analysis.

Classification of maturing status

The maturity of the wild Japanese eel after HCG injection was divided into poor-responsive group and well-responsive group based on skin color and gonadal development as modified from Han et al. (2003a). Briefly, the yellow eels of both sexes have white/gray pectoral fins and yellow/white belly. The poor-responsive male eels have black pectoral fins and light silver/bronze belly with GSI below 1. The well-responsive male eels have black pectoral fins and silver/bronze belly with GSI above 1. The poor-responsive female eels have gray pectoral fins and light silver/bronze belly. The well-responsive female eels have black pectoral fins and silver/bronze belly.

Calculation of morphometric indexes

TL and body weight (BW) were measured to nearest 1 mm and 0.1 g, respectively. Because the difference in size was not significant between left and right pectoral fins or eyes, the left pectoral fin (L_p , ± 0.01 mm), and horizontal (A) and vertical (B) diameters (± 0.01 mm) of the left eye were measured. The weights of the liver (LW, ± 0.01 g), gonad (GW, ± 0.01 g) and digestive tract (DW, ± 0.01 g) were measured after decapitation. Eel sex was determined by visual inspection of the gonads. Five morphometric indexes were calculated (Pankhurst, 1982a; Han et al., 2003a):

$$\text{Ocular index (OI)} = [((A+B)/4)^2 \times \pi / TL] \times 100 \%$$

$$\text{Fin-index (FI)} = [L_p / TL] \times 100 \%$$

$$\text{Gonadosomatic index (GSI)} = [GW / BW] \times 100 \%$$

$$\text{Gut index (GI)} = [DW / BW] \times 100 \%$$

$$\text{Hepatosomatic index (HIS)} = [LW / BW] \times 100 \%$$

Age determination by otolith

The preparation of the otolith for age determination followed the procedure of Tzeng et al. (1994). Briefly, the largest pair of the eel otoliths (sagitta) was extracted, dried at 60° C for 10 min, and embedded in a silicon rubber mould with petropoxy 154 (Palouse Petro Products, U.S.A.). Each otolith was ground and polished from the proximal side with sandpaper until the primordium was revealed. The otolith was then etched with EDTA (5 %, pH = 7.0) for 60 sec to reveal the annulus for age determination.

Data analysis

Significant differences of the mean morphometric indices among different eel groups, and

differences between the initial and subsequent morphological characters of the Japanese eel, were analyzed by analysis of variance (ANOVA) followed by Tukey's HSD multiple range test (Winer, 1971). Differences were considered significant at $\alpha = 0.05$.

Results

Comparison of morphometric indices among eel groups

Visual inspection of the eel gonads after decapitation showed that there were 24 males and 50 females. The morphometric indices of male and female eels on each groups are summarized in Table 1. The total length distribution was shown in Fig. 1. As indicated, eels with TL > 60 cm were all females. The mean initial TL and BW were significantly higher ($p < 0.05$) in the females than in the males (females TL: 522.7 ± 32.2 mm, BW: 150.0 ± 35.9 g; males TL: 472.6 ± 19.1 mm, BW: 103.1 ± 15.2 g). The percentages of the well-responsive eels after HCG treatment were 50 % and 20 % for males and females, respectively (Table 1).

The mean age, TL, BW, and FI were significantly higher in well-responsive eels than in poor ones for both sexes ($p < 0.05$ for all), and the control group showed no significant difference to HCG-injected groups. The well-responsive eels were at least 4 years in males and 5 years in females. The OI of the well-responsive eels were significantly higher than the poor-responsive and control eels for both sexes ($p < 0.05$) (Table 1). The mean GSI was largest in well-responsive eels and smallest in mock-injected eels for both sexes (Table 1). The mean GI of control male eels were significantly higher than the poor- and well-responsive eels ($p < 0.05$), and the mean GI of well-responsive female eels were significantly smaller than the control and poor-responsive ones ($p < 0.05$) (Table 1). The mean CF and HSI showed no significant differences among groups for both sexes (Table 1).

Changes of external morphology on eels injected with HCG

During HCG treatment, the TL, BW, OI and FI were monitored each week, and the change

patterns were calculated (Fig. 2). The mean TL of each groups were stable in both sexes (Fig. 2A, 2B). The mean BW of each groups slightly decreased during treatment (Fig. 2C, 2D). The mean OI of both sexes was higher after HCG treatment, and significant difference was observed in the well-responsive male eels after 4 weeks (Fig. 2E, 2F). No change of OI was found in the control group. The mean FI of each group was also stable for both sexes (Fig. 2G, 2H).

Regression analysis of morphometric indices on each sex

Regression analysis of morphometric indices (OI and DI) on GSI for each sex were shown in the Fig. 3. The regression of OI on GSI in both sexes showed positive correlation (Fig. 3A, 3B), while GI on GSI in both sexes, showed hyperbola-decay correlation (Fig. 3C, 3D). No correlation was observed between HSI and GSI or between FI and GSI of both sexes (Data not shown).

1 Discussion

2

3 The previous study showed that the changes of skin color on the wild Japanese eels were

4 correlated with gonadal development during silvering process (Han et al., 2003a). In the present study,

5 similar phenomenon was also observed after induced maturation. The mock-injected eels of both

6 sexes had unchanged skin color, while the well-responsive groups had more significant characters of

7 silver eels than the poor-responsive ones after HCG treatment. The synchronous changes of skin

8 color and gonadal development are thus consistent either in the wild or in the HCG-injected eels.

9 However, the changes on skin color of HCG-injected males are more pronounced than that of females

10 since all HCG-treated males showed black pectoral fins. Similar trend was also observed in the

11 changes of GSI, OI and GI (Table 1). The shrinkage of digestive tract on poor-responsive males was

12 significant compared to poor-responsive females. The poor-responsive males had 8 folds increase on

13 GSI than control eels, while only 2 fold increase was observed on GSI of poor-responsive females

14 compared to control ones (Table 1). The percentages of the well-responsive eels were markedly

15 higher for males (50 %) than for females (20 %) (Table 1). 2 of the 9 well-responsive males even

16 produced seminal fluid with mature sperm. Consequently, this indicated that the maturation of males

17 is more sensitive and rapid than the females after HCG injection. The better response of the males

18 than females after HCG treatment was found on the artificial propagation test in which most male eels

19 spermiated after 5 injection of HCG, but the female eels usually produced matured oocytes after 12

20 injection (Ohta et al., 1997). This may be due to sex-dependent life-history strategy, with male eels

21 exhibiting a time-minimizing growth strategy by maturing earlier as soon as possible whereas females

22 postponing maturation with a size-maximizing growth strategy to attain higher fecundity (Vøllestad

23 and Johnson, 1986; Helfman et al., 1987; Tzeng et al., 2002; Han and Tzeng, 2006).

Ijiri et al. (1998) reported that the wild silver eel is a better candidate for artificial maturation than feminized or cultured ones because of better development of the gonads. In the present study, the initial gonadal development of well-responsive groups before HCG injection was supposed to be better than the other two eel groups because of their higher initial OI and FI, which had been proved to be positively correlated with the GSI in the wild Japanese eels (Han et al., 2003a). Moreover, the well-responsive eels were larger in mean size and higher in mean age than those of poor-responsive ones, which were in accordance with the previous study on wild Japanese eels that age, size and gonadal development were positively correlated (Han et al., 2001). These results supported that the elder and larger yellow eels, which are supposed to have more developed gonads, are more sensitive to HCG treatment and have better response.

In the previous study, the GSI of the wild Japanese eel was found to be synchronous with the changes of OI and FI before and during silvering process (Han et al., 2003a). In the present study, the mean OI increased in both sexes, especially for the well-responsive males (Fig. 2E, 2F). The synchronization still existed between GSI and OI in both sexes after HCG injection (Fig. 3A, 3B), indicating that the enlargement of eyes during eel silvering may be controlled by hormones of the HPG axis. On the other hand, the FI showed no changes in both sexes after HCG injection (Table 1), different to the linear correlation of FI and GSI in wild silvering eels (Han et al., 2003a). The reason may be due to that both FI and GSI were correlated with body size in the wild eels, resulting in positive correlation between FI and GSI. The enlargement of pectoral fins may not be directly controlled by hormones of the HPG axis.

Marchelidon et al. (1996) found that silver stage European eels with natural fasting presented a more significant degeneration of the alimentary tracts than yellow stage eels with experimental

starvation. Pankhurst and Sorensen (1984) indicated that significant degeneration of digestive tracts was observed in HCG-injected eels. In our previous study, the decrease of GI was synchronous with the increase of GSI during silvering of the wild Japanese eel (Han et al., 2003a). These results inferred the possible effect of HPG axis on eel digestive tract. In the present study, the synchronization was indeed found between GSI and GI after HCG injection (Fig. 3C, 3D), suggesting that the degeneration of alimentary tracts during eel silvering may be also controlled by hormones of the HPG axis.

In Australian eel, injection of high dose 11-ketotestosterone resulted in the increase of HSI in both sexes, and the enlargement of liver was supposed to function for eel migration in both sexes, and also for the production of hepatic yolk precursor molecules, vitellogenin, in the females (Rohr et al., 2001). In the present study, the mean HSIs of the HCG-injected male eels were higher but not significant than the mock-injected control group, and no significant increase was found in the female groups (Table 1). The difference between Australian eel and Japanese eel may be due to species-specific response and differences in hormone treatment. However, the liver color of the well-responsive eels in both sexes were pink red while those of the control ones were dark red. This inferred that the liver did response to HCG injection and performed some role during eel silvering.

In the previous studies, the mRNA expression of GTH α , I- β and II- β significantly increased during eel silvering (Han et al., 2003b). The mean serum testosterone concentrations in both sexes and estradiol in the females were also found to significantly increased during silvering process (Han et al., 2003c). These findings indicated that the HPG axis is active in the process of eel silvering. In female Australian eel, injection of 11-ketotestosterone resulted in gonadal development and silvering-related changes (Rohr et al., 2001). Huang et al. (2001) indicated that combined treatment

of testosterone and cortisol triggered homogeneous silvering-related changes in the European eel. In the present study, HCG injection, which would result in androgen production in the gonad of both sexes (Khan et al., 1987; Sato et al., 2000), induced gonadal development and synchronous changes on body silvering, enlargement of eye size and degeneration of digestive tract. Ikeuchi et al. (1999) found the broad expressions of androgen receptors in eel tissues such as gill, heart, head kidney, spleen, liver, muscle, brain, testis and ovary, providing the possible signaling routes of androgen to affect these organs. These evidences strongly inferred that hormones of HPG axis are the key factor that guide silvering related morphometric changes in the eels.

In conclusion, the synchronous changes in morphology and gonadal development during eel silvering are likely to be under the control of HPG hormones. This synchronization guarantees the simultaneous changes of the morphology and physiology which are necessary for the seawater spawning migration of the eels.

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24

1 **Table 1. Changes of morphometric indices among mock - and HCG - injected eels**

	Mock	HCG - injected		Tukey's HSD
		Poor-responsive	Well-responsive	
Male No.	6	9	9	
Age	3.5 ± 0.4	3.4 ± 0.2	4.2 ± 0.3	P < W
TL	466.3 ± 22.5	447.7 ± 17.4	501.6 ± 14.3	P < W
BW	105.9 ± 16.8	83.1 ± 13.1	121.0 ± 15.0	P < W
CF	0.9 ± 0.1	0.9 ± 0.0	1.0 ± 0.1	M = P = W
OI	5.2 ± 0.8	4.4 ± 0.3	7.3 ± 0.4	M = P < W
FI	5.0 ± 0.5	4.6 ± 0.2	5.3 ± 0.3	P < W
GSI	0.1 ± 0.5	0.8 ± 0.4	7.4 ± 2.6	M < P < W
GI	1.3 ± 0.2	0.5 ± 0.0	0.4 ± 0.1	M > P = W
HSI	1.5 ± 0.2	1.8 ± 0.4	1.7 ± 0.2	M = P = W
Female No.	10	32	8	
Age	5.0 ± 1.0	4.5 ± 0.5	5.7 ± 0.5	P < W
TL	556.9 ± 45.7	486.0 ± 24.2	627.0 ± 42.8	P < W
BW	185.3 ± 36.9	110.7 ± 21.0	294.4 ± 44.7	P < W
CF	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	M = P = W
OI	5.4 ± 0.6	4.2 ± 0.4	6.2 ± 0.5	M = P < W
FI	4.8 ± 0.3	4.4 ± 0.2	5.1 ± 0.2	P < W
GSI	0.4 ± 0.2	0.8 ± 0.2	2.5 ± 0.4	M < P < W
GI	1.4 ± 0.4	1.3 ± 0.2	0.4 ± 0.1	M = P > W
HSI	1.3 ± 0.2	1.2 ± 0.1	1.4 ± 0.2	M = P = W

2 Age: year; TL: total length (mm); BW: body weight (gw); CF: condition factor; OI:
 3 ocular index; FI: fin index; GSI: gonadosomatic index; GI: gut index; HSI: hepatosomatic
 4 index; M: mock-injected eel; P: poor-responsive eel; W: well-responsive eel

Figure Legends

Fig. 1. Number distributions of the total length in the wild male and female Japanese eels.

Fig. 2. Time-course changes of TL (A, B), BW (C, D), OI (E, F) and FI (G, H) during HCG injection for the male (left panel) and female (right panel) Japanese eels. Dark circle: mock-injected eel; empty triangle: poor-responsive eel; dark square: well-responsive eel. * : significant difference compared with initial mean value of the morphometric index.

Fig. 3. Linear regressions of OI (A, B) and GI (C, D) on GSI of the male (left panel) and female (right panel) Japanese eels.

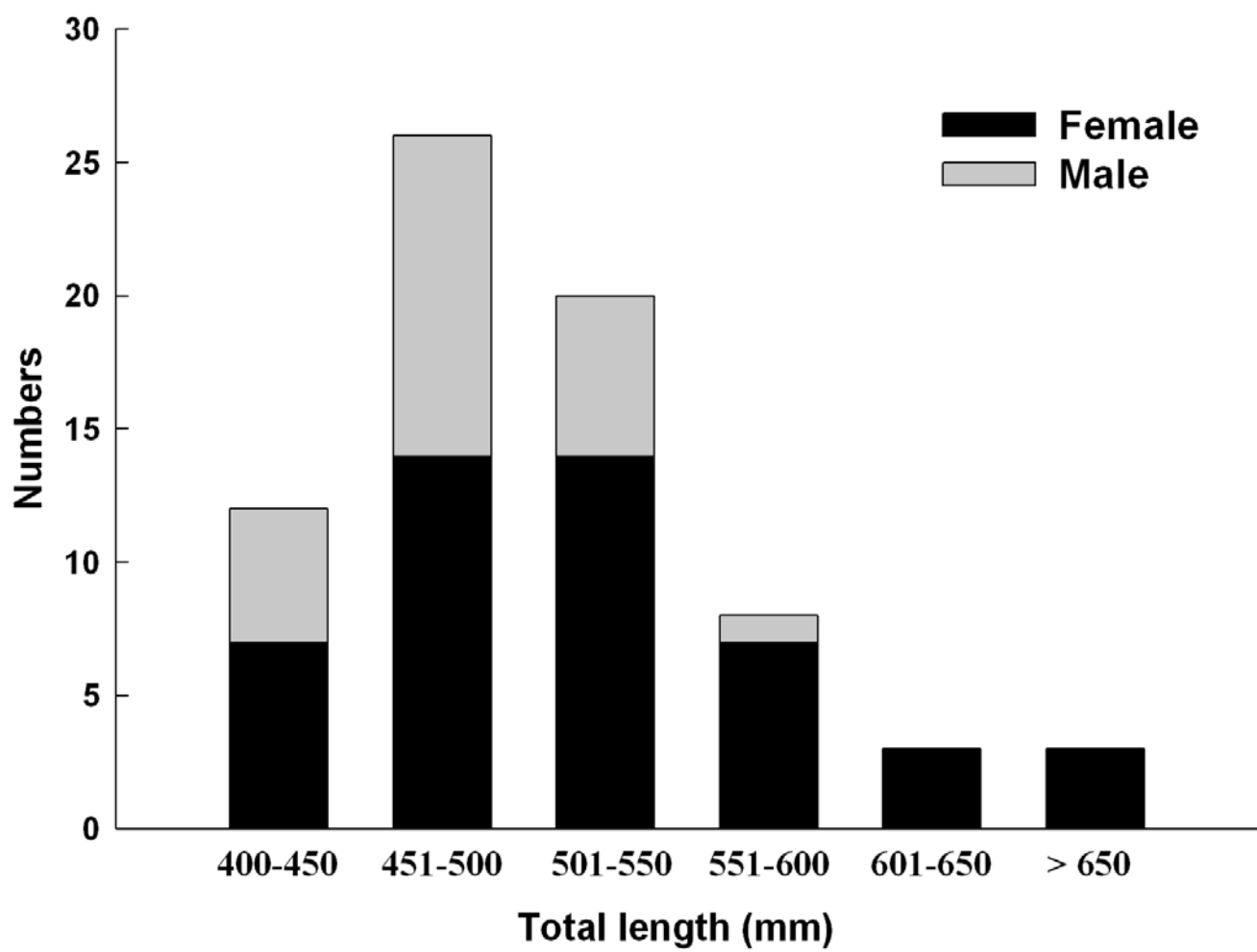


Fig. 1

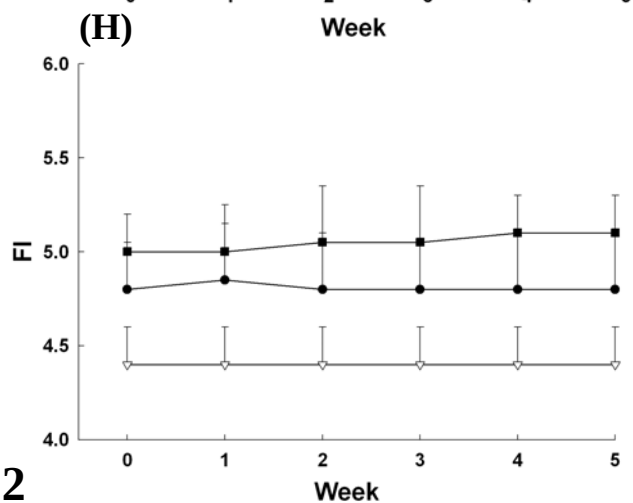
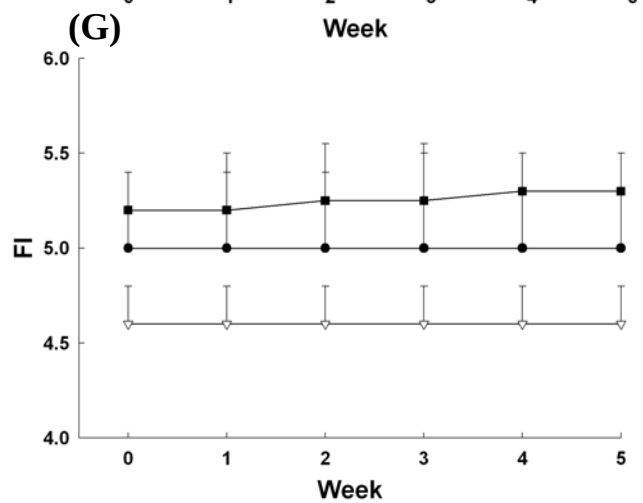
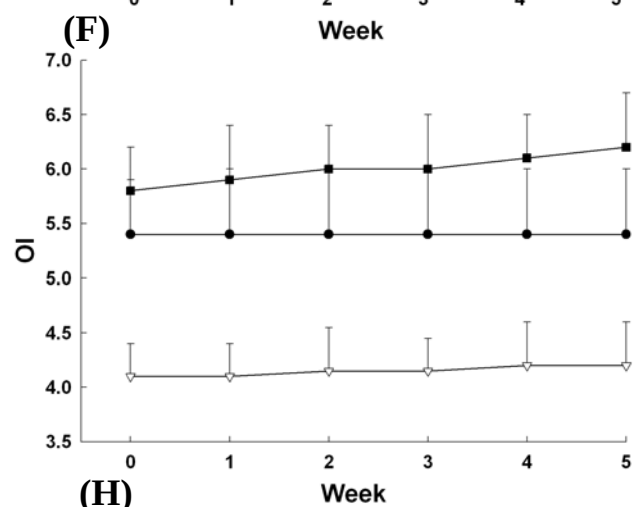
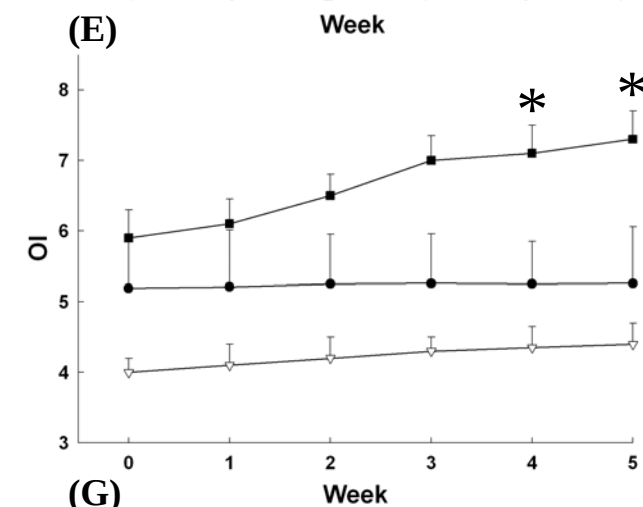
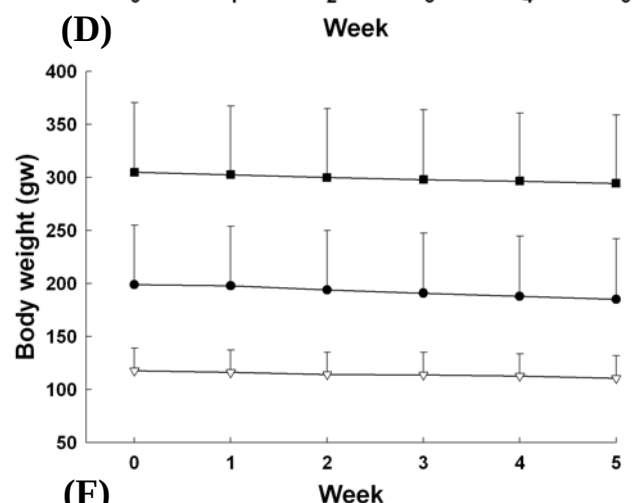
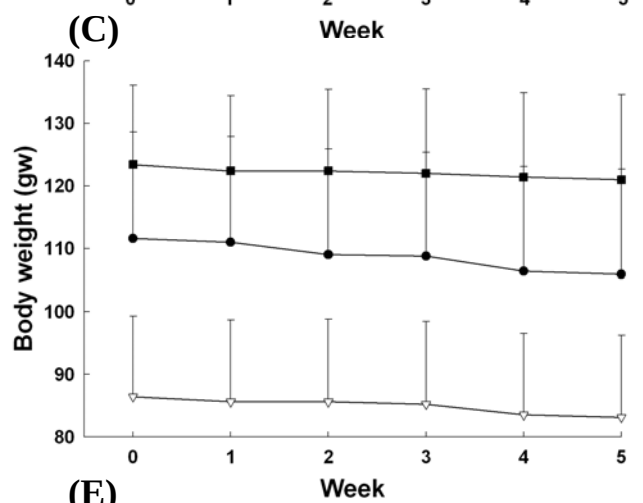
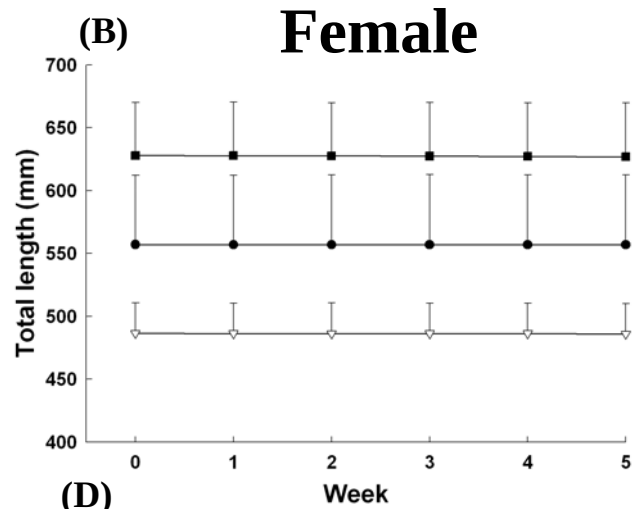
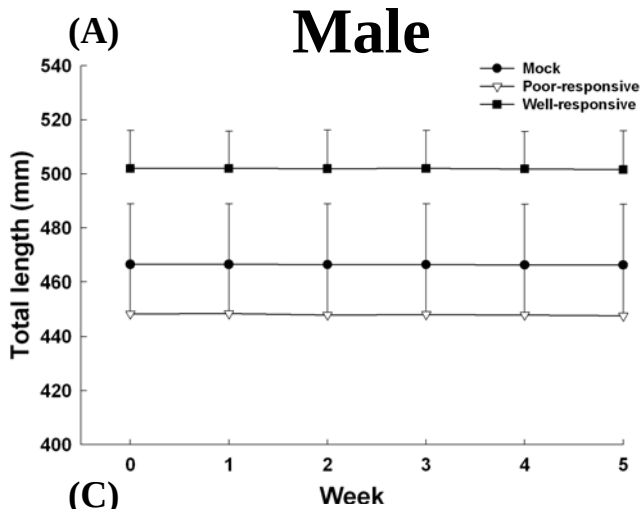
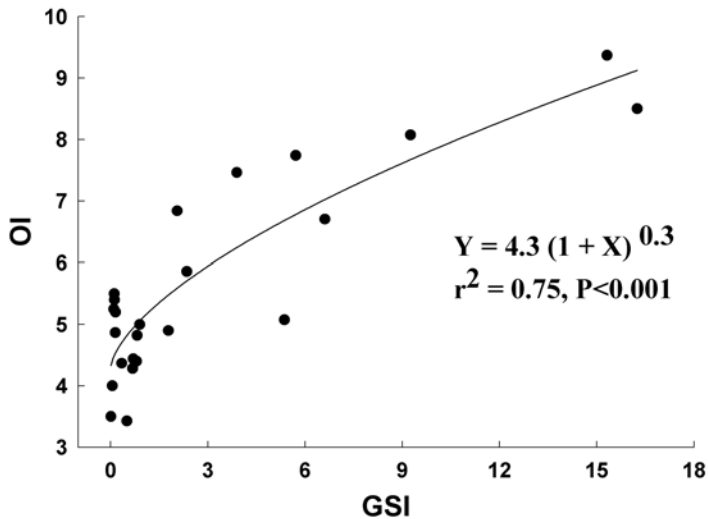
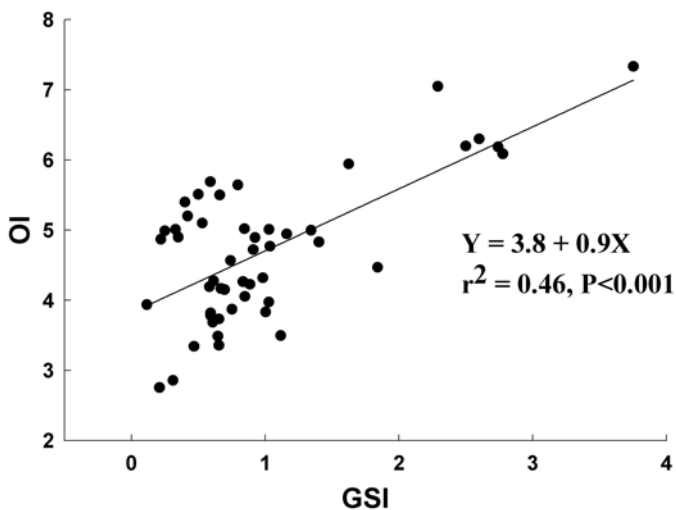


Fig. 2

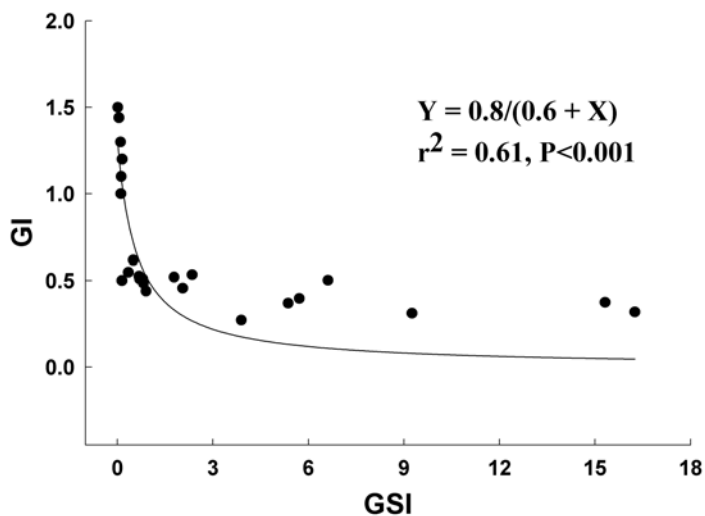
(A) Male



(B) Female



(C)



(D)

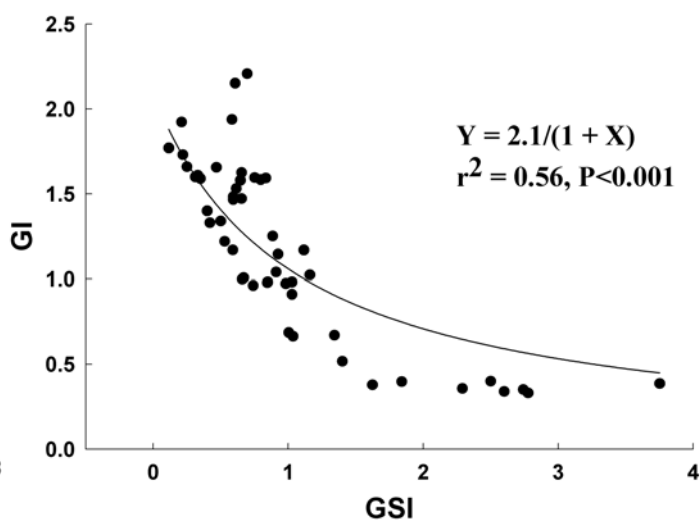


Fig. 3