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Biological Aspects of *Channa limbata* (Cuvier, 1831) in Ta Bo - Huai Yai Wildlife Sanctuary, Phetchabun Province, Thailand

(Aspek Biologi *Channa limbata* (Cuvier, 1831) di Suaka Hidupan Liar Ta Bo - Huai Yai, Wilayah Phetchabun, Thailand)

KAN KHOOMSAB* & SUWIT WANNASRI

ABSTRACT

The biological aspects of Channa limbata *were studied between November 2013 and October 2014. A total of 346 fish specimens, 185 male and 161 female, were collected from Ta Bo, Huai Yai Wildlife Sanctuary, Phetchabun Province, Thailand. Specimens range from 7.3 - 17.2 cm in length with body weight 8 - 31 g; sex ratio between males and females was 1: 0.7. The length (L), weight (W) relationship for mixed sexes was* $W = 0.2064 L^{1.85}$ *(*R^2*=0.90). Gonadosomatic indices for males and females were measured monthly and varied from 0.21 - 0.65% and 1.96 - 3.74%, respectively. Condition factors for males and females ranged between 0.54 - 2.20 and 0.58 - 2.72, respectively, with fecundity range 956 to 4,652 eggs in females. Fecundity (F) to weight relationship was* $F = 189.53 W^{0.59}$ *(*$R^2 = 0.71$*) and fecundity to length relationship was* $F = 68.82 L^{1.15}$ *(*$R^2 = 0.77$*). The ratio between the intestine length and total length was 1:2, indicating that* C. limbata *was a carnivorous feeder. Analysis of the stomach contents gave 84% insects and 16% aquatic weed. These results can be applied to conserve efforts to prevent the extinction of* C. limbata *in protected areas.*

Keywords: Biological aspects; Channa limbata; *fecundity; gonadosomatic index; length- weight relationship*

ABSTRAK

Aspek biologi bagi Channa limbata *telah dikaji antara November 2013 dan 2014 Oktober. Sejumlah 346 spesimen ikan, 185 jantan dan 161 betina telah diambil dari suaka hidupan liar Ta Bo - Huai Yai, wilayah Phetchabun, Thailand. Julat spesimen adalah daripada 7.3 - 17.2 cm panjang dengan berat badan 8 - 31 g; nisbah jantina antara jantan dan betina adalah 1: 0.7. Hubungan panjang (L), berat badan (W) untuk jantina campuran adalah* $W = 0.2064 L^{1.85}$ *(*R^2*=0.90). Indeks gonadosomatik bagi jantan dan betina telah dikira secara bulanan dan berbeza-beza masing-masing daripada 0.21 - 0.65% dan 1.96 - 3.74%. Faktor keadaan bagi jantan dan betina adalah dalam lingkungan antara 0.54 - 2.20 dan 0.58 - 2.72, masing-masing dengan julat kesuburan 956 untuk 4,652 telur betina. Kesuburan (F) kepada hubungan berat adalah* $F = 189.53 W^{0.59}$ *(*$R^2 = 0.71$*) dan kesuburan kepada hubungan panjang* $F = 68.82 L^{1.15}$ *(*$R^2 = 0.77$*). Nisbah antara panjang usus dan jumlah panjang adalah 1:2 yang menunjukkan bahawa* C. limbata *adalah merupakan pembekal karnivor. Analisis kandungan perut memberikan 84% serangga dan rumpai akuatik 16%. Keputusan ini boleh digunakan untuk usaha pemuliharaan dalam mencegah kepupusan* C. limbata *di kawasan pemeliharaan.*

Kata kunci: Aspek biologi; Channa limbata*; hubungan panjang-berat; indeks gonadosomatik*; *kesuburan*

INTRODUCTION

Snakeheads (family Channidae) are air-breathing freshwater fishes from two genera: *Channa* native to Asia, Malaysia and Indonesia and *Parachanna* that is endemic to tropical Africa. However, the taxonomy of Channidae is not universally agreed, with leading authorities on snakehead systematics currently recognizing 26 species of *Channa* and three species of *Parachanna* (Courtenay & Williams 2004). The genus *Channa* is distributed from southeastern Iran and eastern Afghanistan eastward through Pakistan, India, southern Nepal, Bangladesh, Myanmar, Thailand, Laos, Malaysia, Sumatra, Indonesia, Vietnam, Korea and China and northward into Siberia (Courtenay & Williams 2004; Goswami et al. 2006). *Channa limbata* is one of the smallest species in the family Channidae, commonly termed the red-tailed snakehead. It is considered rare as it is found only in shallow forest streams and waterfalls close to mountainous terrain in Thailand.

Knowledge of some quantitative aspects is important in studying fish biology. Length and weight are standard data used in fish programs to estimate growth rates (Cherif et al. 2008). Pauly (1993) studied the length-weight relationships (LWRs) of a fish to predict weight from length measurements made in the yield assessment. In addition, previous research concerning Channidae concluded that the length-weight and length-length relationships of *C. diplogramma*, *C. marulius*, *C. striata*, *C. punctatus* and *C. punctata* followed by allometric growth (Ali et al. 2013; Datta et al. 2013; Kashyap et al. 2014). Hannifar et al. (2006) investigated length-weight relationships of *Channa punctata* from Western River in which the study showed no significant difference (p>0.05) in the LWR as a function of sex.

Furthermore, gonadosomatic index (GSI) and fecundity studies are useful for making total population estimates, population dynamics or productivity. GIS were studied in *C. marulius* and *C. ghachua* which peak value of GSI was observed only once in the month of indicating only one spawning period in *C. marulius* i.e. from June to August while *C. punctatus,* GSI reaches maximum in August (Ghanbahadur et al. 2013; Prasad et al. 2011; Tiwari et al. 2014). *C. punctatus* was the highest values of GSI of in rainy season (Kapil et al. 2011).

The former measures gonad mass relative to total body mass and is a good indicator of sexual maturity, while the latter denotes egg laying capacity and refers to the number of ripe eggs produced during one spawning season (Nandikeswari & Anandan 2013). Accordingly, information on reproduction is useful for fishery management and conservation (El-Ganainy 2010; Hossain et al. 2015, 2012; Maithya et al. 2012; Njiru et al. 2006).

To date, there is still lack of evidences available regarding the biological aspects of *C. limbata* in Thailand to facilitate management and planning for a sustainable natural population. Therefore, this study investigated the length-weight relationships, sex ratios, gonadosomatic indices, condition factors, fecundity and stomach contents of *C. limbata* in the Ta Bo-Huai Yai Wildlife Sanctuary, Muang District, Phetchabun Province, Thailand. The results can be used as a basis for further research on breeding to promote conservation and sustainable natural population.

Materials and Methods

Sampling Site

Samples of *C. limbata* were collected from Ta Bo-Huai Yai Wildlife Sanctuary, Muang District, Phetchabun Province, Thailand (16°22’42.2”N 101°17’21.4”E). This area is abundance with *C. limbata*.

Sampling and Laboratory Analysis

C. limbata specimens were collected overnight (18.00 to 06.00) from streams 0.5 to 20 m wide and 6 to 25 cm depth once a month for 12 months from November 2013 to October 2014 using a five basket fish trap. The samples were stored in ice before transportation to the biological laboratory at the Faculty of Science and Technology, Phetchabun Rajabhat University. The number of fishes collected each month is listed in Table 2. A total of 346 *C. limbata* specimens including 185 males and 161 females were measured. Standard lengths, total lengths and body proportions were measured using a slide caliper (Mitutoyo blade type). Body weight was recorded on a digital balance (E-scale DYB-300). All samples were preserved in 10% formalin and the taxonomic study followed the method of Villéger et al. (2010).

Sex Ratio

The fish were dissected and the sex characteristics of males and females were determined by examining the gonads (Ramez 2005). Statistical calculations for the sex ratio were performed using the Chi-square test (Rosner 2011) under the assumption that the proportion of males and females was 1:1.

Length-Weight Relationship

The *C. limbata* specimens were weighed with a precision balance at a resolution of 0.01 g. Total lengths were measured by a ruler at resolution of 0.1 cm and the relationship between weight and total length was determined using the following formula from Jin et al. (2015):

$$W = a L^b \tag{1}$$

which was transformed into a logarithmic equation as:

$$\log W = \log a + b \log L \tag{2}$$

where W is the weight (g); L is the length (cm); and a and b are constants.

The log-transformed (2) is a linear regression model. After calculating the relationship equation and the coefficient of determination (R^2), further examination was conducted to determine whether the equation explained the fluctuations of the dependent variables (y-axis).

Gonadosomatic Index

The specimens were cleaned and weighed. The stomach of each fish was then cut out to weigh the seminal vesicle and the ovaries. The weights were used to calculate the GSI following the method of Gaikwad et al. (2009) as follows:

$$\text{GSI} = \frac{\text{weight of reproductive organ}}{\text{fish weight}} \times 100 \tag{3}$$

Monthly means of the GSI were compared to observe changes and to estimate the time of year when the reproductive organs attained their highest development.

Condition Factor

Monthly data regarding the weights and lengths of the fishes were used to calculate the condition factor using the method of Jin et al. (2015) as follows:

$$K = 100(W/L^3) \tag{4}$$

where K is the condition factor; W is the weight (g); and L is the total length (cm); the factor of 100 is used to bring K close to a value of one.

FECUNDITY-LENGTH AND WEIGHT RELATIONSHIP

The lengths of female *C. limbata* that were ready to lay eggs or whose eggs were in the maturity stage according to Nikolsky (1963) were measured. Their ovaries were then weighed and the eggs randomly counted. To do this, all the eggs were weighed and approximately 10% of the ovaries were randomly weighed. The eggs that were randomly chosen for counting were cleaned and the egg tissues were cut off. The ovaries were then cut lengthwise, opened and soaked in Gilson's fluid (100 mL 60% alcohol, 880 mL water, 15 mL 80% nitric acid, 18 mL glacial acetic acid and 20 g mercuric chloride). After soaking for over 24 h, the bottle was agitated to separate the eggs and the fluid was drained off. The leftover tissues were then removed, water was added and the bottle was shaken well and left for sedimentation. The water and the soft parts of the eggs were then removed and the eggs were filtered and drained before counting. The data were used to calculate the relationship between the length and weight of the fishes and the fecundity, according to Agbugui (2013) as follows:

$$F = a L^{b} \text{ or } \log F = \log a + b \log L \quad (5)$$

$$F = a W^{b} \text{ or } \log F = \log a + b \log W \quad (6)$$

where F is the number of eggs; L is the total length (cm); W is the weight (g); and a and b are constants.

STOMACH CONTENTS

Stomach contents were studied using the occurrence method (Hyslop 1980; Jamabo & Maduako 2015), cutting them open to examine the undigested material. The samples were analyzed using a low-power microscope. This was a basic study that compiled and recorded the food groups by examining and categorizing the types of food found in the stomachs. The results were displayed as percentages.

$$\text{Frequency of occurrence} = \frac{X}{Y} \times 100 \quad (7)$$

where X is the number of stomachs where each food item was present and Y is the total number of stomachs.

RESULTS AND DISCUSSION

TAXONOMIC CHARACTERISTICS OF *CHANNA LIMBATA*

The red-tailed snakehead, *C. limbata,* is a species of freshwater fish in the Channidae family. Its shape is similar to other species of fish in the family, but the red-tailed snakehead is rounder and bigger. The body color ranges from light brown to dark blue, with polka-dot patterns or dark spots. The stomach is a light color. The bases of the pectoral fins have patterns of four - six dark lines. The dorsal fin, the anal fin and the caudal fin are gray or bluish with orange or light edges.

The body color is pale and it is almost impossible to see any pattern, while the dorsal, anal and caudal fins are opalescent bluish green. The edge of the caudal fin has an orange stripe. Fish in this family have a slim cylindrical shape, protruding wide mouths, big eyes, canine teeth, large flat heads that appear round like a snake, round bodies, long dorsal and anal fins, rounded caudal fins, large pectoral fins, small pelvic fins and cycloid scales. Fish in the striped snakehead group have a respiratory suprabranchial organ that appears as a red narrow opening in the pharynx.

C. limbata has a long, cylindrical body with a large mouth and sharp teeth. The enlarged scales on top of the head with eyes located far forward are similar to the scale patterns and eye positions of snakes. The largest ever recorded snakehead was almost six feet long. *C. limbata* is the smallest species in the Channidae family and ranges from the south of China to Myanmar, Thailand, Laos, Malaysia and Indonesia (Amilhat & Lorenzen 2005; Song et al. 2013; Ward-Campbell & Beamish 2005; Wijeyaratne 1994). Adult *C. limbata* are only 15 to 20 cm in length. *C. limbata* is considered rare as it is found only in shallow forest streams and waterfalls close to mountainous terrain. Most of *C. limbata* are classified as *C. gachua*, which is a similar species found in India. However, in reality, these two fish types are quite different and subject to ongoing academic documentation and discussion. Morphological measurements of the average lengths of the sample specimens collected are shown in Table 1.

TABLE 1. Morphological measurements of average lengths of *Channa limbata*

Morphometric characteristics (n = 346)	Average length (cm)
Body standard length	10.97
Body depth	1.62
Caudal peduncle minimal depth	1.28
Caudal fin depth	1.12
Caudal fin surface	0.82
Distance from insertion of pectoral fin to bottom of body	1.41
Body depth at level of pectoral fin insertion	1.67
Pectoral fin length	1.90
Pectoral fin surface	1.35
Head depth along vertical axis of eye	1.23
Eye diameter	0.41
Distance from center of eye to bottom of head	0.96
Distance from top of mouth to bottom of head along head depth axis (measured with electronic caliper)	0.51
Body width	2.35
Mouth depth	2.16
Mouth width	2.01

SEX RATIO

Random catching of *C. limbata* during the year produced 346 fish in total, 185 males and 161 females (Table 2). The overall sex ratio of males to females was 1:0.7. Study of the sex ratio assumed that the number of males was equal to females (1:1) at a reliability level of 95% and the mean Chi-square value for the whole year was 0.34 with a mean significance of 0.64. Therefore, the sex ratio of *C. limbata* equaled 1:1 in accordance with the assumption. It was impossible to clearly differentiate the males and females from their appearance and even during the spawning season changes in reproductive organs could not be easily observed unless the stomach was pressed lightly, causing the eggs to ooze out. The testis and ovaries were investigated by dissection. The results regarding the sex ratio agreed with other researchers who also found more males than females (Benghali et al. 2014).

LENGTH-WEIGHT RELATIONSHIP

Fish lengths varied between 7.3 and 17.2 cm and the weights varied between 8 and 31 g. The average weight was 18.33±5.70 g and the average length was 10.97±1.89 cm. The analysis of the relationship is displayed by (8) and (9):

$$W = 0.2064\ L^{1.85} \quad (8)$$

$$\log W = 1.85 \log L - 0.68\ (R^2 = 0.84, n = 346) \quad (9)$$

R^2 was 0.84, indicating that the length-weight of *C. limbata* had a significant relationship (Figure 1).

When only the 185 male *C. limbata* were considered, the average weight was 17.94±0.51 g and the average length was 10.82±1.81 cm. This relationship is displayed by (10) and (11):

$$W = 0.2202\ L^{1.83} \quad (10)$$

$$\log W = 2.72 \log L - 11.60\ (R^2 = 0.80, n = 185) \quad (11)$$

R^2 equaled 0.80, indicating that the length-weight of male *C. limbata* had a significant relationship (Figure 2).

When only the 161 female *C. limbata* were considered, the average weight was 18.21±5.75 g and the average length was 11.09±1.89 cm. This relationship is displayed by (12) and (13):

$$W = 0.1854\ L^{1.89} \quad (12)$$

$$\log W = 1.89 \log L - 0.73\ (R^2 = 0.84, n = 161) \quad (13)$$

R^2 equaled 0.84, indicating that the length-weight of female *C. limbata* had a significant relationship (Figure 3).

The logarithmic regression equation for the length-weight relationship of *C. limbata* was smaller than the cubic value ($b < 3$) for both males and females, indicating an allometric growth pattern. An allometric growth pattern was also found in *C. diplogramma* and *C. marulius* (Ali et al. 2013). Kashyap et al. (2014) recorded an overall isometric growth pattern ($b = 3.01$) in *C. punctatus* from the Gomti River, India. The weight of *C. punctatus* was almost the cube of its length and showed negative allometric growth ($b < 3$) in males and positive allometric growth ($b > 3$) in females. Thus, females grew longer than the males. The length-weight relationship with regards to allometry is essential to understand the basic growth pattern of a species. Among the allometric relationships, the length-weight relationship (LWR) of fishes can indicate the species' status in an environment and characterize patterns of growth (Ali et al. 2013; Froese 2006).

ESTIMATION OF GONADOSOMATIC INDEX

Study of the GSI of female *C. limbata* showed values between 1.96 and 3.74% increasing from June to October. For male *C. limbata*, GSI values were 0.21 - 0.65% (Figure

TABLE 2. Number and ratio of male and female *Channa limbata* collected monthly between November 2013 and October 2014

Month/ year	Number of males	Number of females	Total	Expected value	Male:female ratio	Chi-square	Sig.
Nov 2013	9	7	16	8	1:0.7	0.25	0.61
Dec 2013	12	15	27	13.5	1:0.8	0.33	0.56
Jan 2014	21	15	36	18	1:0.7	1.00	0.31
Feb 2014	18	12	30	15	1:0.6	1.20	0.27
Mar 2014	10	7	17	8.5	1:0.7	0.52	0.46
Apr 2014	18	15	33	16.5	1:0.8	0.27	0.60
May 2014	16	15	31	15.5	1:0.9	0.03	0.85
Jun 2014	11	14	25	12.5	1:0.7	0.36	0.54
Jul 2014	6	8	14	7	1:0.7	0.00	1.00
Aug 2014	16	18	34	17	1:0.8	0.11	0.72
Sep 2014	25	18	43	21.5	1:0.7	0.00	0.92
Oct 2014	23	17	40	20	1:0.7	0.02	0.86
Total	185	161	346				
Mean average				13.1	1:0.7	0.34	0.64

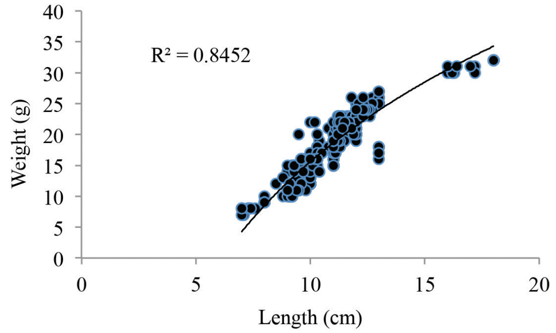

FIGURE 1. Length-weight relationship of *Channa limbata* with no gender discrimination

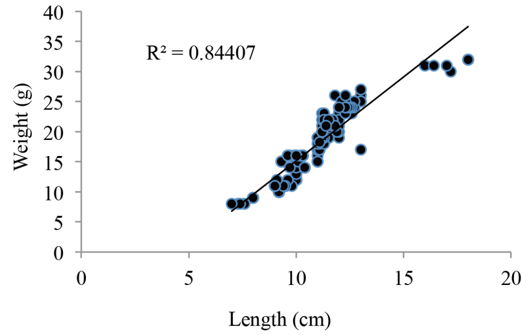

FIGURE 3. Length-weight relationship of female *Channa limbata*

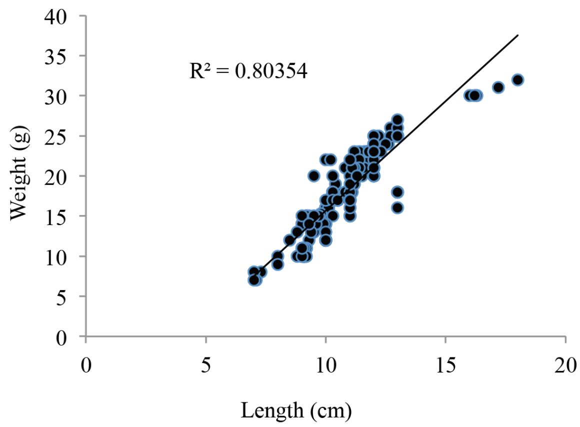

FIGURE 2. Length-weight relationship of male *Channa limbata*

4). These results agreed with the condition factors. GSI values were higher in females than males. Tiwari et al. (2014) reported a peak in GSI value for *C. marulius* in May, with a spawning period from June to August. Kapil et al. (2011) found that the GSI of *C. punctatus* was highest during the rainy season for both males and females. Mishra (1991) determined the highest GSI at 6.8 in June for *C. gachua* (Dwarf Snakehead). Several methods have been used to study the spawning season and the spawning grounds of aquatic animals. In tropical areas, the stage of development of the sex organs, the distribution of eggs and the survey of eggs and hatchlings assumes that when aquatic animals enter their spawning season, the weights of their sex organs will increase and peak before laying eggs. The results from this study showed that the monthly variation in GSI for *C. limbata* was highest during the rainy season. This concurs with the spawning theory of freshwater fishes in tropical areas, indicating that most freshwater fishes, including snakeheads, lay eggs during the rainy season.

CONDITION FACTOR

The condition factor of female *C. limbata* was between 0.58 and 2.72 and for males between 0.54 and 2.20 (Figure 5). Monthly variation in the condition factor indicated that the spawning season of *C. limbata* was during the rainy season (May - October). The results showed that the condition factor was higher in females than males. The condition factor is useful for the examination of aquatic animal health (Hossain et al. 2015). It gives an indication of food availability and the status of the reproductive systems, which reflects physical and biological circumstances. Fluctuations in the condition factor results from feeding conditions, parasitic infections and physiological factors.

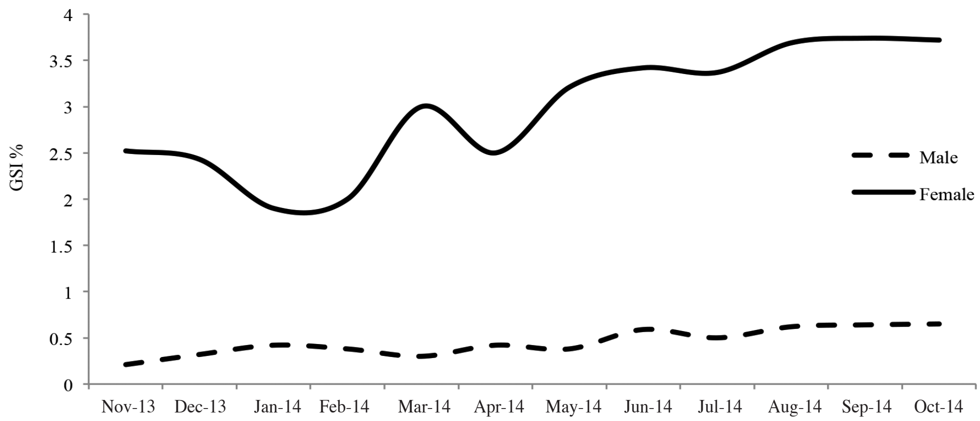

FIGURE 4. Gonadosomatic index of *Channa limbata*

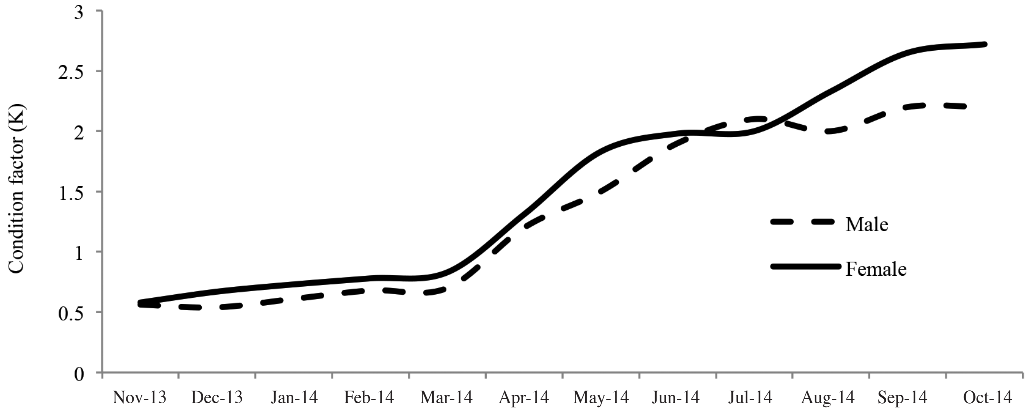

FIGURE 5. Condition factor of *Channa limbata*

The condition factor also reflects changes in food reserves and is, therefore, an indicator of the general fish condition (Datta et al. 2013; Islam et al. 2013).

This study determined that the spawning season of *C. limbata* was during the rainy season, with development of the testis and eggs peaking in July and August 2014. It is possible to prevent extinction and develop *C. limbata* into a future economic species with artificial insemination of the eggs by the sperm of the fish. One problem which arose during the research was the difficulty in accurately differentiating the gender from the appearance alone. However, *C. limbata* is a rare species in its natural habitat of clean, running streams in mountain valleys and in-depth research and study are necessary to prevent future extinction.

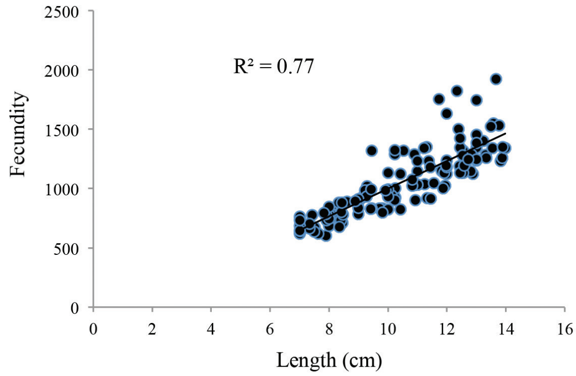

FIGURE 6. Relationship between fecundity and length

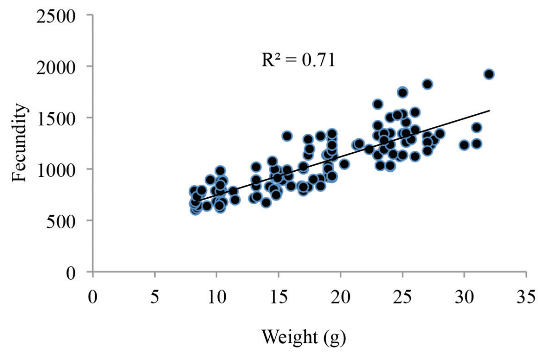

FIGURE 7. Relationship between fecundity and weight

FECUNDITY RELATIONSHIP

For the female *C. limbata,* the average length was 11.09±1.89 cm and the average weight was 18.21±5.75 g. The relationship between the length, weight, and number of eggs of female *C. limbata* was determined using (14) and (15):

$$F = 68.82\, L^{1.15} \, (R^2 = 0.77, n = 161) \tag{14}$$

$$F = 189.53 W^{0.59} \, (R^2 = 0.71, n = 161) \tag{15}$$

R^2 values were calculated as 0.77 and 0.71, indicating how the fecundity of *C. limbata* related to length and weight (Figures 6 and 7). The fecundity of the eggs refers to the number of ripe eggs or the eggs that are about to ripen, in the ovary before laying. Previous studies determined that the fecundity varied according to the size and weight of the fish. This explanation is usually expressed as a power function. The results showed 956 to 4,652 eggs in females. Mishra (1991) estimated that the fecundity varied between 2,539 and 7,194 in 15 mature specimens of *C. gachua*, ranging from 13.4 to 17.2 cm in length. Relationships between fecundity and length as well as fecundity and body weight were linear. However, the fecundity estimation must consider a variety of attributes, including size at first sexual maturity, duration of spawning season, daily spawning behavior and spawning fraction, when evaluating the commercial potential of fish stocks (El-Drawany 2013; Hossain et al. 2012). A relationship between length, weight, and fecundity for the Channidae family was reported by Gaikwad et al. (2009) for *C. gachua*, Islam et al. (2013) for *C. striatus* and Widodo et al. (2013) for *C. gachua*. Knowledge of fecundity is useful for the imposition of adequate regulations for the conservation of this threatened species (Hossain et al. 2012). Relative fecundity, as the number of eggs per unit weight, is commonly used as an index of fecundity. The fecundity of an individual female also varies according to many factors including age, size, species, food availability and season.

STOMACH CONTENTS

Eighty-four percent of the food found in *C. limbata* stomachs was insects and sixteen percent was small pieces of plant material. Therefore, it was concluded that *C. limbata* are carnivorous. The ratio between intestine length and total length was 1:2, and this also indicated that the fish is a carnivorous feeder. Carnivorous fishes generally have shorter intestines than their omnivorous and herbivorous cousins (Qin & Fast 1996). The food items of snakeheads consist mainly of mouse, tadpole, fish, prawn, other crustaceans, terrestrial insects, detritus, smaller ephemeropterans, ipterans, hymenoptera, odonata, lepidoptera and some mosquito larvae (Bolaji et al. 2011; Lee & Peter 1994; Ward-Campbell & Beamish 2005). However, the gut contents only indicate what the fish has fed on recently. Accurate descriptions of fish diets and feeding habits are important to understand the trophic interactions in aquatic food webs. Fish diets represent an integration of many important ecological components that include behavior, condition, habitat use, energy intake and other inter/intraspecific interactions.

CONCLUSION

This study considered *C. limbata*, the smallest member of the Channidae family found in Thailand. Linear regression was used to determine the length-weight relationship. The results indicated an overall allometric growth pattern, with GSI values and the condition factor increasing during the rainy season and lowest in the winter and summer seasons. The breeding season from March to October agreed with the spawning theory that freshwater fishes in tropical areas, including snakeheads, lay eggs during the rainy season. The relationships between fecundity, total length, body weight and number of eggs were found to be significant. The results can be used to initiate breeding programs for *C. limbata* to protect this threatened and endangered Thai freshwater fish species.

ACKNOWLEDGEMENTS

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Kan Khoomsab*
Biology Program
Faculty of Science and Technology
Phetchabun Rajabhat University, Phetchabun
Thailand

Suwit Wannasri
Education Science Program
Faculty of Science and Technology
Phetchabun Rajabhat University, Phetchabun
Thailand

*Corresponding author; email: topkan13@hotmail.com

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