Body Size and Morphological Characteristics in sex determination of Humphead Wrasse *Cheilinus undulatus* in captivity

B L Tan¹, F F Ching¹* and S Senoo¹,²

¹ Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia
² Shirahama Aquaculture Research Institute, Kinki University, 3153, Shirahama, Wakayama 649-2211, Japan
Email: cfuifui@ums.edu.my

Abstract. This study was aimed to identify the sex of humphead wrasse, *Cheilinus undulatus* kept in captivity based on body sizes and morphological characteristics. There were 65 tails of *C. undulatus* broodstocks reared in the captivity throughout six months of research period and fed daily with enrich prey fish (Sardinella sp.). Growth parameters and morphological characteristics such as presence of eyeline, hump on head and genital papillae, of the *C. undulatus* were carried out. These findings revealed in captivity, female of *C. undulatus* (n=13) attained an average body weight of 2.78 kg and total length 47.10 cm meanwhile male of *C. undulatus* attained larger body weight (14.62 kg) and total length exceeding size of female (89.30 cm). *C. undulatus* performed an isometric growth pattern (b = 2.9487) with condition factor of above 0.2. Morphologically, female was identified with the noticeable alleviation of the hump on its head and the visibility of eyeline behind its eyes which was not seen in male. This study concluded sex of *C. undulatus* in captivity is differentiated through body size and morphological features and take longer time in sex transition, thus balanced sex ratio for good broodstock management can be controlled to develop appropriate breeding protocol.

1. INTRODUCTION
Humphead wrasse is scientifically known as *Cheilinus undulatus*. Common names are Mãori wrasse, Napoleon wrasse, so mei, and also called mameng across Asia pacific region [7]. This species is the largest member in Labridae family. It could reach to the maximum size exceeding 2 m in total length and 190 kg in body weight in the wild [2,3,6]. Study on wild adult of *C. undulatus* has blue-green body color with large scales and a yellow posterior margin to the caudal fin, while small individual has pale body color with black markings [2]. *C. undulatus* is observed with a thick, fleshy lips and a hump that forms on its head above the eyes which will becomes more prominent as the fish ages. The significant appearance of male *C. undulatus* was observed on its body color which ranged from bright electric blue to green, a purplish blue or relatively dull blue or green [7]. As reported by Sadovy *et al.*, (2003), *C. undulatus* changing its sex from female to male or known as protogynous hermaphrodite. In its natural habitat, female *C. undulatus* generally reaches sexual maturity at 5 years of age and 35 to 50 cm in average total length [13]. Then, it changes its sex to male at 9 years of age.
C. undulatus is an expensive and highly sought-after fish in Southeast Asia [13]. As a result, there is increment for international live trade of C. undulatus recorded from 58 tonnes in year of 2000 to 138 tonnes in year of 2006 [6]. The major exporter countries of C. undulatus are Indonesia, Malaysia and Philippines [13]. CITES Appendix II (2004) has listed C. undulatus as the first endangered coral reef fish which makes it a very important aquaculture candidate. Mass production of C. undulatus under artificial condition has never been easy and hindered by insufficient understanding on reproductive biology of C. undulatus specifically on sex differentiation and gonad maturation. The objective of this study was to identify sex through body size and morphological characteristics and its maturation status of C. undulates kept in captivity.

2. MATERIALS AND METHODS

The experiment was conducted in the Marine Hatchery of Borneo Marine Research Institute in Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia. A total of sixty-five tails of C. undulatus ranged from 5.42±3.26 kg of body weight (BW) and 62.10±10.68 cm of total length (TL) were used in this study. All of the tested C. undulatus were tagged with microchip by using an injector prior to the start of research. Then, they were transferred to a 150 tonnes cylindrical HDPE (High Density Poly Ethylene) circular tank of seawater.

The water quality throughout the research was maintained at 6.37 ± 0.02 mgL-1 for dissolved oxygen, pH of 6.98 ± 0.11 and temperature of 28.53 ± 0.36 °C. C. undulatus were fed until satiation at 0800 with enriched prey fish (Sardinella sp.) with 0.1 ml of pure cod liver oil and squid (Sepia sp.). Feeding to C. undulatus was done six times a week. All of the C. undulatus were measured throughout the study from December 2012 to May 2013 and continued at June 2017. First, a concentration of 10 μL-1 of anesthesia TRANSMORE tranquilizer (alpha – methylquinoline) (Nika) was diluted into 500 L water volume and C. undulatus were left for 3-5 minutes. Once C. undulatus was fully anaesthetized, identification of C. undulatus was checked via tag on their bodies by using MPR mini portable reader. Three main parts of the body of C. undulatus was observed to identify the sex including genital papillae, head shape, and eye line of each brood fish. Availability of oocyte and sperm of C. undulatus were examined by cannulation method [14] and samples were immediately preserved in Bouin solution for histology observation. Samples were later on embedded in paraffin, sectioned at 5 μm prior to staining with hematoxylin and eosin. The gonadal stage of female were classified as one of three female phase stages (O1, primary-growth stage (developing stage) and 30 to 110 μm in diameter; O2, cortical-alveolus stage (mature stage) and about 220 μm; O3, vitellogenic stage (spawning stage) with more than 400 μm oocyte diameter), and intersex- transitional stage (I, the presence of spermatogonia and/ or spermatocytes and spermatids in the ovarian tissue), two male phase (M1, the presence of spermatocytes and spermatids (developing stage); M2, with spermatozoa (mature stage)) [16].

Then, the weight of C. undulatus was taken by using XK3190 – A12 electronic balance (China). C. undulatus was quickly placed on a wood board with measuring tape of 100 cm at bottom before its morphological measurements were recorded. The recorded morphology measurement included total length (TL), standard length (SL), head length (HL), body weight (BW), body width (BWt), and head width (HWt). Growth parameters were calculated for growth performance [1]. The length-weight relationship was plotted and analyzed in log graph using the equation as below.

\[ SGR (\%) = 100 \times \left( \frac{\text{ln Weight initial} - \text{ln Weight final}}{\text{day}} \right) \]

\[ \text{Condition factor, } k = 100 \times \left( \frac{\text{W}}{L^3} \right) \]

\[ \text{Survival rate (\%) = Final total of fish / initial total of fish} \times 100 \]

\[ \text{Length-weight ratio, } r = \frac{L}{W} \]

\[ \text{Length-weight relationship} = (\log W = b \log L + \log a) \]
3. RESULTS

3.1 Sex identification based on body weight and total length

A total of 13 tails of female were found with BW ranging from 1.00 kg to 7.18 kg and total length of less than 67.50 cm (Figure 1). Among the female found, the minimum BW was recorded at 1.00 kg with TL of 34.90 cm. Meanwhile, 1 tail of male was found with BW approximately more than 7.18 kg (in this case 14.62 kg) and TL of more than 67.50 cm (in this case 89.30 cm). Meanwhile, 51 tails of *C. undulatus* were remained undifferentiated.

![Figure 1 Distribution of body weight (g) and total length (cm) of male, female and undifferentiated humphead wrasse in captivity](image_url)
The relationship of body weight and length were shown in the length-weight relationship graph in Figure 2. The equation was log transformed to estimate the parameter ‘b’. The value of ‘b’ was 2.9487; while r² value was observed as 0.9907.

3.2 Growth parameters
The findings were clearly revealed that the SGR for male of *C. undulatus* was negative at -1.0% meanwhile females was found with an increasing SGR of 11.5%. Condition factor (k) for male was 0.24; while 0.23 for female. The length-weight ratio for both sexes for male and females was 16.80 and 4.63, respectively. The survival rate of *C. undulatus* was 100 % for both sexes.

| Parameters                  | Male  | Female |
|-----------------------------|-------|--------|
| Specific growth rate (SGR) (%)| -1.0  | 11.5   |
| Condition factor (k)        | 0.24  | 0.23   |
| Length-weight ratio         | 16.80 | 4.63   |
| Survival rate (%)           | 100   | 100    |

3.3 Sex identification through morphological observations
The findings had revealed clearly that male of *C. undulatus* displayed an elongated genital papillae (Figure 3a); while female of *C. undulatus* was observed with rounded genital papillae with the presence of a small egg hole (Figure 3b).
On the other hand, the hump of male was easily distinguishable by the presence of relatively higher and bigger hump (Figure 4a). Unlike the hump on male, female of *C. undulatus* was observed with lower and smaller bump (Figure 4b). Alleviation of the hump was seen more obvious on female *C. undulatus*.

Meanwhile, female was identified with the visibility of eyeline behind its eyes which was not seen in male *C. undulatus*. All the female, ranged from 1.00 kg to 7.18 kg, was observed with a visible eyeline which was absence in male *C. undulatus* (Figure 5a). Another noticeable line was found in front of its eye in female *C. undulatus* (Figure 5b).
Figure 5 (a) Absence of eyeline in male *C. undulatus*, (b) presence of eyeline in female *C. undulatus*

Table 3.2: Morphological observation of male and female, humphead wrasse

| Parameters                  | Male                  | Female                                      |
|-----------------------------|-----------------------|---------------------------------------------|
| Structure of genital papillae | Elongated             | Rounded with a small egg hole               |
| Hump                        | Higher and bigger     | Lower and smaller                           |
| Eyeline Visibility          | Absent                | Noticeable adjacent to the female’s eye     |

3.4 Gonad observations

Gonad, in this case, two ovaries were obtained from two tails of the sampled *C. undulatus*. Gonadosomatic index (GSI) of the sampled ovaries were 0.78 (Ovary 1) and 0.12 (Ovary 2) respectively (Figure 6a and 6b). The first gonad weighed 0.0595 kg (length, 4.4 cm) was obtained from a female of *C. undulatus* with body weight of 0.76 kg and total length of 29.8 cm. While, the second gonad was 0.0107 kg (length, 4.0 cm) was obtained from another female of *C. undulatus* with body weight of 0.91 kg and total length of 36.3 cm. No testes were collected in this study.
Figure 6  The characteristic of (a) Ovary 1, (b) Ovary 2 of female humphead wrasse in captivity

Table 3.3: Gonad growth of ovaries obtained from two different female humphead wrasse reared in captivity

| Parameters                  | Ovary 1 | Ovary 2 |
|-----------------------------|---------|---------|
| Female Weight (kg)          | 0.76    | 0.91    |
| Female Total Length (cm)    | 29.8    | 36.3    |
| Gonad Weight (g)            | 59.5    | 10.7    |
| Gonad Length (cm)           | 4.4     | 4.0     |
| GSI                         | 0.78    | 0.12    |

3.5 Histological observations of oocytes
Histological observation on the first gonad was observed with oocyte of primary-growth stage (O1), cortical-alveolus stage (O2) and vitellogenic stage (O3) (Figure 7a and 7b). On the other hand, oocyte in second gonad was identified as ovary with oocyte of primary-growth stage (Figure 8a and 8b). A nucleus, in oval shape, can be seen in O1 stage of oocyte, while O2 also observed with an enlarged nucleus inside the enlarged oocyte. O3 is distinguishable with many cortical alveoli presented in oocyte.
Figure 7 (a) Histological analysis reveals the gonad maturation status at O1 with the presence of primary-growth stage and O2, a cortical-alveolus stage in female humphead wrasse in captivity.

Figure 7 (b) Histological analysis reveals the gonad maturation stage at O3 with the presence of vitellogenic stage.

Figure 8 Histological analysis on gonad maturation status of second female humphead wrasse (a) and (b)

4. DISCUSSION

4.1 Sex identification based on body weight and total length

Based on present research, the number of females of *C. undulatus* were more than male. It is not surprise to see more female by taking *C. undulatus* is a hermaphrodite species and experience sex changing from female to male as they reach maturity of age. Besides, it showed that there were possibilities that the largest female would transform into a male fish if there was no male fish among them. According to Sadovy *et al.* (2003), there are possibilities that one of the females will dominantly and transform into a male to control the harem when multiple females kept together. Same findings were found in mostly marine fish species which the largest female that can increases its fitness by changing its sex when male fish was absence in a harem [8]. Within a few days, the largest female in the harem becomes a dominant
male and takes over the missing male's function [10]. This pattern is common in coral reef fishes, such as parrot fish, wrasses, and groupers.

In comparison to other reef fishes, some groupers are gonochoristic, whereby they display a reproductive strategy with two distinct sexes. FAO (2004) reported that the determination of age and growth of *E. aeneus* by using annular rings on scales documented a 9.7 kg of fish was 8 years old also the females mature at 5 to 7 years with 1.5 to 3.0 kg, 50 to 60 cm total length. It was suggested that the sex change occurs at 10 to 13 years with 6 to 15 kg, 80 to 110 cm total length. There were also several findings showing that most groupers are sexually mature within 2 to 6 years but some of the larger species may take longer to mature such as *E. fuscoguttatus*, which matures at about 9 years [15]. As compared to sea bass, females at the same age collected at the South China Sea were discovered to be heavier and longer than the males as stated by Yue *et al.*, (2012). In case of black sea bass, it changes sex to male at around two to five years old.

4.2 Growth parameter
Besides the body weight, relationship between the length and weight were determined in present study and used as a parameter to further understand the growth dynamics of a fish population as stated by Dutta *et al.* (2012). However, the present study showed a poor growth performance by taking the weight did not showed any increment in line with the length. In terms of growth, female fish showed a relatively higher SGR value compared to male fish and this might be caused by the large energy used by male to compensate the needs in reproductive development rather than growth. In this study, male was also kept in smaller cage to avoid been attack by the aggressive female. Similar study reported by FAO (2004), whereby when male fish are kept in cage, they tend to maintain a sedentary life style as they aged and grow bigger. Hence, less space was provided would lead to stress level and low feed consumption. However, both male and female fish attained 100% survival rate and it reflects the hardiness to adapt in artificial environment in captivity. Other than survival and growth, condition factors are important to describe and compare the wellbeing of a fish in terms of condition or fitness [4]. Imam *et al.* (2010) suggested that when the condition factor is less than 3, the fish shows a negative allometric growth pattern. It was believed that as the condition factor increased, the size of the fish also increased since it grows proportionally in all directions. It was described that as the condition factor increased, as well as the sexual maturation of a fish [4]. There are some factors affected the value of condition factor such as stress, sex, season, availability of feeds and water quality parameters as reported by [12].

4.3 Sex identification through morphological observations
For morphological features observations, only the alleviation of hump on the head, presence of eyeline and the size of genital papillae showed major differences in both sexes. Compared to common marine aquaculture species such as groupers and sea bass, only dominant male *C. undulatus* appears to display a hump on its head. This characteristic was not observed in female *C. undulatus* throughout this research. Several findings shows that as the time for releasing gametes gets nearer, the ephemeral coloration patterns were noticed in a few grouper species and also in *C. undulatus* [11]. In rock hind grouper, they displayed a dark band below the eye in the tuxedo pattern, which appeared as a very important display for the males and were only displayed by dominant males [11]. Besides than the eyeline and hump on its head, the size and structure of its genital papillae also highly differentiated. Male have smaller size of genital papillae compared to female, probably responsible for the difficulty in obtaining sperm of male *C. undulatus*. Further more, genital papillae for males are made of multiple thicker layers compared to the structure of females. Meanwhile, the sex of other marine aquaculture fish species such as sea bass can only be determined easily in mature fish. Male sea bass of the same age are generally smaller with slender and narrower body depth compared to the females. During spawning season, the milt can be observed at
the genital opening if slight pressure is applied on the abdomen of the mature male while the female can be recognized from the big soft round belly with the red-pink papilla extruding out at the urogenital aperture. Nevertheless, none of these identification characteristics were observed in mature C. undulatus.

4.4 Gonad observation

Besides identification of sex through body weight and morphological features, internally, the sexes of most marine animals can be determined by gonad examination. C. undulatus, can be either synchronous in which individuals possess both ovarian and testicular tissue or sequential in which individuals change sex [5]. In present research, mature female was found with the presence of oocyte of primary-growth stage (O1), cortical-alveolus stage (O2) and vitellogenic stage (O3). Meanwhile, these stages were absent in the mature inactive female except for primary-growth stage. The developing oocytes progressed from a cortical alveolus (perinucleolar) and vitellogenic stage through vitellogenesis and ovulation, with concomitant increases in ovarian mass and oocyte diameter [5]. However, no gonad examination that had been done for male fish and it is suggested to be done for future studies. During sexual transition, the oocytes will degenerate making the spermatogonia proliferate and the ovary is transformed into a functional testis [7]. For transitional sex fish, it was found out by Kline et al., (2011) that there were yellow-brown bodies were discovered in gonadal sections, potentially indicating remnants of atretic oocytes and recent sex change. Generally, the intersex fish would reduce its gonad size and darker colour was observed compared to the ovary and testes [11]. However, the sea bass male would spawn at least once before changing sex [11]. Only short period is consumed for the transition process to happen and it may not occur in all individual of sea bass fish [17]. Due to reorganizing of the gonad morphologically and in terms of steroidogenic capacity, the time for gonad sex to change is longer than behavioural change at least for eight days for C. undulatus [11]. Meanwhile, as found by Chandrasekhara & Krishnan, (2007) that the lowest size of transitional individual was observed in E. diacanthus at 27.5 cm total length where the transitional gonads appeared similar to maturing female and male stages with the presence of early stages of spermatogonial proliferation and degenerating oocytes in the ovary. In another finding, the evidence of protogynous hermaphroditism in groupers were the absence of small males with the presence of atretic oocytes and brown bodies in the testes of mature males reported by Edward et al., (2003). Grouper gonad development undergoes transition from ovary to intersexual gonad and then to testis, where the primordial germ cells and different stages of gametic cells during oogenesis and spermatogenesis are synchronously observed in the transitional gonads [8]. For mature fish, it was discovered by Edward et al., (2003) that the smallest and largest observed adult female individuals of the Hawaiian grouper were 32.8 and 97.7 cm in total length with the smallest female whose ovaries contained hydrated oocytes was 49.2 cm in total length meanwhile the smallest and largest adult male fish encountered were 75.3 and 110.3 cm in total length and five transitional fish ranged from 76.0 to 91.3 cm in total length.

From this study, it can be concluded that the sex of C. undulatus is identified based on their body sizes and morphological characteristics. Female are relatively smaller compared to male and can be determined easily with the presence of noticeable hump on head meanwhile female can be identified by the presence of visible eyeline adjacent to the eyes.

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