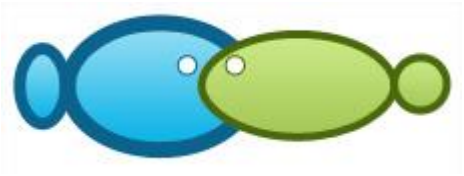

Length-weight relationship, relative condition factor, morphological and endoscopic assessments for sex differentiation of cultured Amur catfish, *Silurus asotus*, broodstock

[1,2]Hanis A. Mohmin, [1]Norfazreena M. Faudzi, [1]Fui F. Ching, [1,2]Shigeharu Senoo

[1] Borneo Marine Research Institute, Malaysia Sabah University, Kota Kinabalu, Sabah, Malaysia; [2] UMS-Kindai Aquaculture Development Centre, Kindai University, Shirahama, Wakayama, Japan. Corresponding authors: F. F. Ching, cfuifui@ums.edu.my

Abstract. The study aimed to investigate the length-weight relationship (LWR), the relative condition factor (RCF), the sex differentiation, through morphological and endoscopic evaluation of the Amur catfish (*Silurus asotus*) cultured in captivity. *S. asotus* were examined after two different culture periods: at 19 months (140 fishes) and at 48 months (341 fishes). The regression coefficient *b* values in the population of *S. asotus* cultured for a 19 months period were separately calculated for male (2.95) and female (2.92) specimens, showing a negative allometric growth, while combined sexes (3.05) showed a positive allometric growth. After the 48 months period, *b* values of the LWR were 2.91 for male, 2.31 for female and 2.67 for both sexes, all exhibiting a negative allometric growth. Despite the negative allometric growth observed in the LWR results, the RCF from both cultured groups was in the range from 1.00 to 1.17. The sex of this species was easily differentiated by the presence of split caudal fins in the male and non-split fins in the female in 48 months. Endoscopic examination of the reproductive organs of the *S. asotus* further confirmed these results. This study concluded that the broodstock of *S. asotus* can be considered to be in good health when cultured in captivity and that sex determination by morphological and endoscopic examinations is effective for better broodstock management.
Key Words: external morphology, relative condition factor, selection technique.

Introduction. The Amur catfish, *Silurus asotus*, is a freshwater species of fish from the family of *Siluridae*. Other common names for the *S. asotus* include "Far Eastern catfish", "Japanese catfish" and "Chinese catfish" (Huckstorf 2012; Sariat et al 2020). This species of catfish is carnivorous and widely distributed in Japan, China, Korea, Taiwan and Vietnam (Kim et al 2015). *S. asotus* is known for its organoleptic and nutritious qualities. The *S. asotus* had been introduced in Malaysia four years ago through research collaborations between Universiti Malaysia Sabah and Kindai University, Japan (Sariat et al 2020). This species of catfish has the potential to become an equally good aquaculture species in comparison to other catfish species, such as the "African catfish" (*Clarias gariepinus*), "Bagrid catfish" (*Mystus nemurus*) and the "River catfish" (*Pangasius hypophthalmus*).

Broodstock selection is an essential part of general aquaculture practice that guarantees the success of the production cycle of each fish species. The basis for obtaining ideal broodstock for production depends largely on the growth and fitness conditions of the broodstock of known sex. In addition, the length-weight relationship (LWR) provides useful information about fish species in a given geographic region (Morato et al 2001). Evaluation of LWR is a common method to determine the general health of cultured aquatic animals (Lim et al 2013). It is a very useful tool that reflects the well-being of the species and provides important information about the fish and their culture conditions (Olurin et al 2006; Lim et al 2013; Kumar et al 2014).

Fish condition analysis has become a standard procedure in fish population management, to measure the health of individuals and groups (age or size) (James et al 2015). Condition factors have generally been described as the well-being or robustness of an individual fish species, estimated by comparing the weight of fish of a given length to a standard weight (Blackwell et al 2000). However, the study of LWR and of the relative condition factor (RCF) of *S. asotus* broodstock in tropical climates and captivity is not well documented. Moreover, the Gonadosomatic index (GSI) is one of the most important parameters in the study of reproductive biology of fish species. It is also used to assess the maturity of ovaries (Nandikeswari et al 2014) and serves as an indicator of reproductive seasonality in fish species (Shafi 2012). Sex determination based on the external morphology of fish species is very important to reduce handling stress while selecting broodstock. The shape of genital papillae is one of the most reliable methods to determine the sex of fish in aquacultures. The male of *C. gariepinus* has an elongated genital papilla, while the female has a rounded genital papilla. However, it has been difficult to determine the sex of *S. asotus* by mere observation of the genital papillae because their shape is undifferentiated. Accordingly, difficulties in sex determination based on external morphology may increase stress in the broodstock selection, resulting in unsuccessful seed production. Therefore, the present study aims to differentiate sex of *S. asotus* via endoscopic method.

Apart from the sex differentiation, this study also aimed to provide information on the LWR, RCF and GSI to assess the overall health and reproductive conditions of *S. asotus*. This objective is fundamental for the development of strategies for managing the broodstock of *S. asotus*, e.g., to understand the optimum of breeding *S. asotus* and to determine the selection techniques for sexually mature male and female.

Material and Method

Rearing protocols. This study was conducted in the fish hatchery, Borneo Marine Research Institute, Universiti Malaysia Sabah, Malaysia. *S. asotus* from two different culture periods were investigated, i.e., 19 months (140 fish) and 48 months (371 fish). The fish used in this study were obtained through artificial seed production and the culture conditions were similar for both 19 and 48 months of culture periods. As the fish grew larger, they were transferred to a 20-ton fiberglass tank and cultured in a closed system with aerated green water. Water exchange was about 20%, and the bottom of the tank was cleaned every two days. The water was maintained at a specified temperature of 29.33±1.73°C, a dissolved oxygen of 5.10±0.27 mg L^{-1} and a pH of 8.34±0.05. The fish were hand-fed until the satiation level with marine pellets (obtained from Leong Hup Feedmills (M) Sdn Bhd; crude protein: 45%; crude lipid: 8%).

Fish body measurement. *S. asotus* were handled carefully and harvested from its culture tank. They were initially anesthetized (Transmore, NIKA, Malaysia) to minimize handling stress for fish. Growth measurements of the body weight (BW) and total length (TL) each of the *S. asotus* were taken from both culture groups using scale weight and measurement board.

LWR and condition factors. The length-weight relationship and the condition factors of the fishes in both culture groups were calculated as follows (Froese 2006):

$$\text{Length-Weight Relationship (W)} = aL^b$$

Where:
W - body weight in (g);
L - total length in (cm);
A - intercept;
b - slope.

The logarithm of the said formula was as follows (Garcia 2010):

$$\text{Log } W = \text{Log } a + b \text{ Log } L$$

The linear regression graph was plotted using an MS-Excel computer program to estimate the values of the constant (a), slope (b), and the coefficient of correlation (r) of the LWRs and the significant differences. The growth condition of the fish was categorized using *b* values; where $b=3$, the fish attained an isometric growth; where $b<3$, the fish indicated a negative allometric growth; and where $b>3$, the fish indicated a positive allometric growth (Lim et al 2013).

The RCF was calculated according to the formula of Le Cren (1951), as follows:

Relative condition factor (Kn) = W (Observed weight, g) / W' (Expected weight, g)

Where:
W' - the expected weight of the fish individual (aL^b) with the earlier estimated *b* value;
Kn - 1 or more is an indication that the fish has achieved the expected growth.

Gonadosomatic index (GSI). A total of 10 tails of *S. asotus* in both culture groups were then dissected to obtain the fish gonad. The gonadosomatic index (GSI) was calculated with the following formula (Tagarao et al 2020):

Gonadosomatic Index (GSI) = (gonad weight (g) x 100) / body weight (g)

Morphology and endoscopic assessment. A total of 20 tails of *S. asotus* broodstock from the 48-month culture period were examined. The fish sampling and measurement procedures performed were similar to fish sampling procedures. The TL and BW were measured and photographed on the whiteboard, while the morphology features were observed and recorded. Attention was directed to the characteristic of the caudal fin. The expected male or female were determined based on the percentage of their split caudal fin or non-split caudal fin, the qualitative findings were converted into quantitative data as follows:

Expected male or female = (SCF or NSCF/FS) x 100

Where:
SCF - no. of fish with split caudal fin;
NSCF - no. of fish non-split caudal fin;
FS - total no. of fish sampling.

Endoscopic assessment of the fish gonads was conducted by performing a minor surgical incision on the body of the fish. A customized flexible inspection device consisting of a fiberscope and otoscope attached to a mobile phone was inserted into the body of the fish, and the image was projected on the screen of the mobile phone.

Statistical analysis. The regression coefficient, r of LWR of male, female and both sexes were analyzed using the ANOVA analysis of variance, through the regression analysis in MS Excel computed at a significance level $a=0.05$. The reproductive performance parameters between the male and female for both cultured groups were compared through the Student's t-test. The SPSS v.27.0 software (IBM, Armonk, NY, USA) was used to perform the statistical analysis at a significance level of $p<0.05$.

Results

LWR and condition factor. The LWRs of the linear regression graph of the male, female and total *S. asotus* cultured in 19 months and 48 months are shown in Figure 2 and Figure 3, respectively, while the estimated parameters of the LWR for both cultured groups are summarized in Table 1 and Table 2. The LWR data obtained from this study

showed different values for male, female and both sexes of *S. asotus*. Specifically, for *S. asotus* from the 19 months culture period (Figure 2), the equation for the male species was y=2.9483x-2.088, the equation for the female was y=2.9154x-2.010 and the equation for both sexes was y=3.0218x-2.1943. The correlation coefficient equation r was 0.96, 0.97 and 0.97 and the determination coefficient was R^2=0.9149, 0.9423 and 0.9435, for the males, females and both sexes, respectively.

For *S. asotus* from the 48 months culture period (Figure 3), the equation of LWR for males, females and both sexes was found to be y=2.9058x-2.088; y=2.3102x-0.983 and y=2.6661x-1.593, respectively. The correlation coefficient assessed at 48 months was r=0.83, 0.86 and 0.89 and the determination coefficient, R^2, was 0.7913, 0.6853 and 0.7351 for males, females and both sexes. It was significantly high at α =0.05, indicating that the calculated values of ln a and the slope (b) were statistically significant.

The calculation (at a 95% confidence interval) of the b value for each population is shown in Table 1 and Table 2. The growth performance of *S. asotus* cultured in captivity for 19 and 48 months was compared using a Student's t-test with a significance level of p<0.05, as shown in Table 3. During the 19months culture period, there were significant differences in the body length of both sexes: 34.08±4.10 cm for males and 39.39±4.41 cm for females; t=7.15; p=<0.001.

There was also a significant difference in body weight between males (283.84±130.87 g) and females (453.76±142.71 g); where t=-7.13 and p=<0.001. The same conditions were observed for the 48 month culture period. There was a significant difference in TL and BW for both sexes. Average TL for males was 41.15±4.4 cm and for females it was 43.99±5.59 cm (t=-5.42; p=<0.001). Average BW showed a significant difference between males (504.01±178.99) and females (682.01±245.33) g, where t=-7.92 and p=<0.001. The RCF of *S. asotus* cultured in captivity for 19 and 48 months are shown in Table 3 and 4, respectively. Overall, the *S. asotus* RCF ranged between 1.00 and 1.17 in each cultured period.

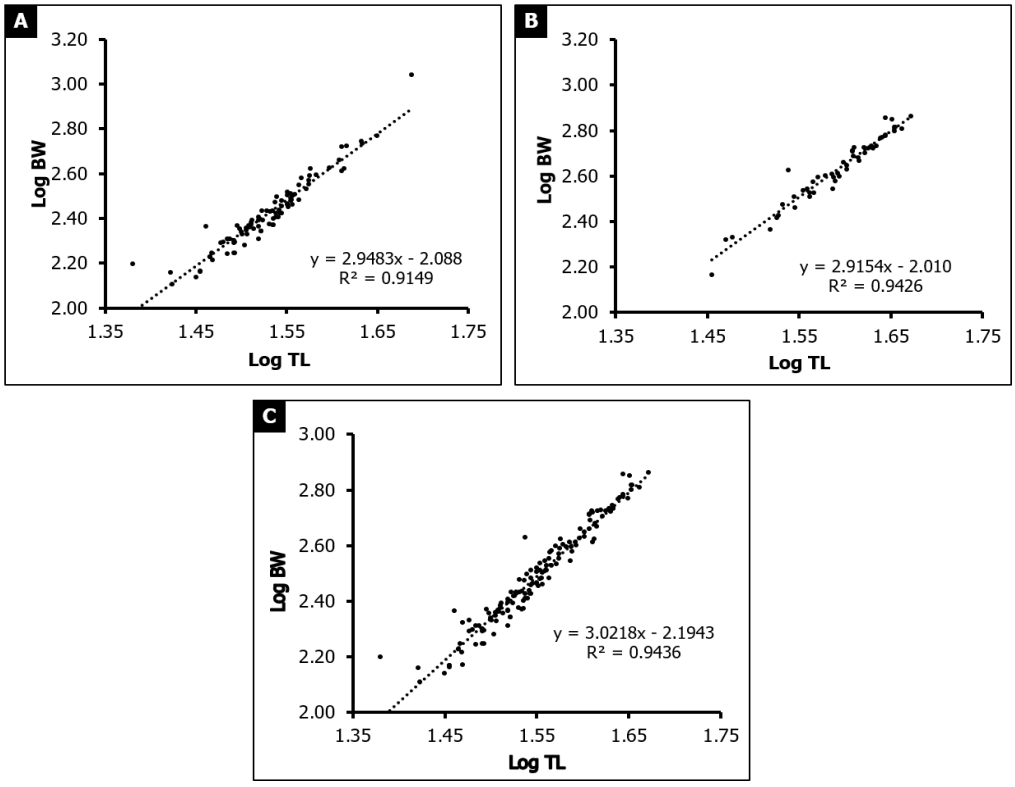

Figure 2. Length-weight relationship of *Silurus asotus* culture in captivity for 19 months period; (A) Males (n=90); (B) Females (n=50); (C) Both sexes (n=140).

Table 1

Estimated parameters in the length-weight relationship of *Silurus asotus* broodstock, for a 19 months culture period, through regression analysis

Category	*N*	*Body weight (g)*		*Total length (cm)*		*Ln a*	*b*	*S.D. of b*	*95% of C.I*	*r*
		Min	*Max*	*Min*	*Max*					
Male	90	128.0	1100.0	26.5	38.7	-2.088	2.9483	0.0958	2.76-3.14	0.96*
Female	50	147.0	729.0	28.5	46.9	-2.015	2.9183	0.1042	2.71-3.13	0.97*
Both sexes	140	128.0	1100.0	26.50	48.7	-2.194	3.0463	0.0634	2.92-3.17	0.97*

N-number of specimens; Min-minimum; Max-maximum; a-intercept of regression line; b-slope of regression line; S.D.- standard deviation; C. I.- confidence interval; r-correlation coefficient;* significant at α=0.05.

Table 2

Estimated parameters in the length-weight relationship of *Silurus asotus* broodstock, for a 48 months culture period, through regression analysis

Category	*N*	*Body weight (g)*		*Total length (cm)*		*Ln a*	*b*	*S.D. of b*	*95% of C.I*	*r*
		Min	Max	Min	Max					
Male	177	170.0	1080.0	28.1	53.1	-2.008	2.9058	0.1128	2.68-3.12	0.89*
Female	194	147.0	1515.0	27.6	57.4	-0.908	2.3102	0.1130	2.09-2.53	0.83*
Both sexes	371	147.0	1515.0	26.50	48.7	-1.593	2.6661	0.0833	2.50-2.83	0.86*

N-number of specimens; Min-minimum; Max-maximum; a-intercept of regression line; b-slope of regression line; S.D.- standard deviation; C. I.- confidence interval; r-correlation coefficient;* significant at α=0.05.

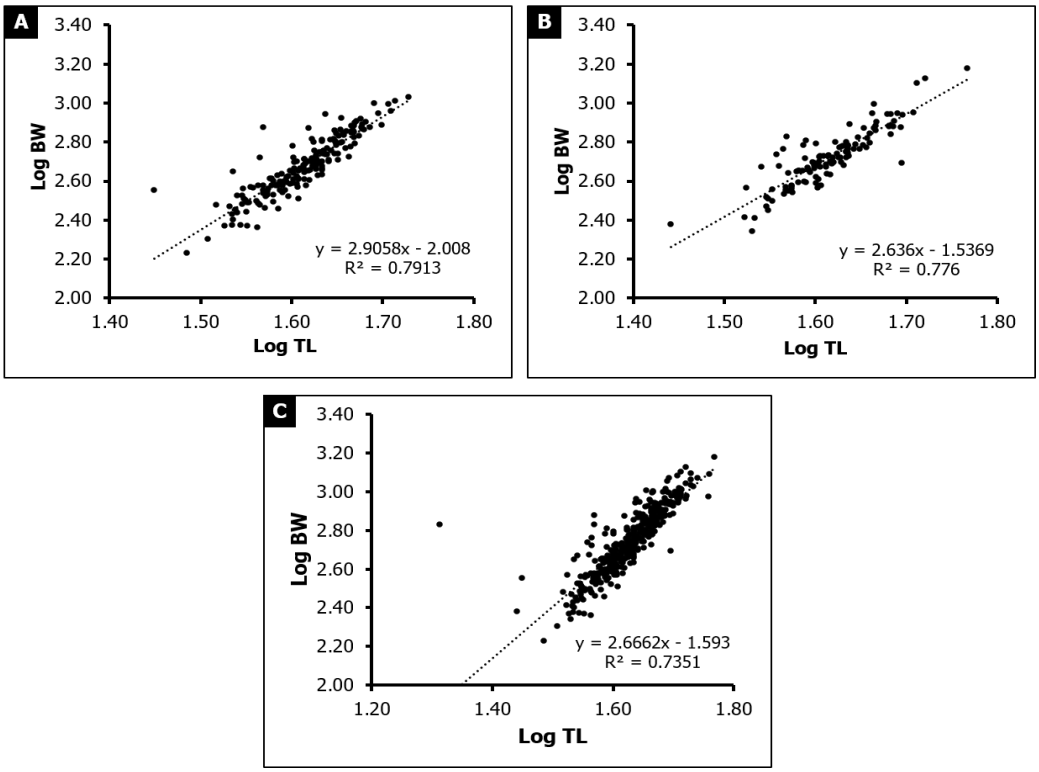

Figure 3. Length-weight relationship of *Silurus asotus* cultured in captivity, for a 48 months period; (A) Males (n=177); (B) Females (n=194); (C) Both sexes (n=371).

Table 3

The observed, expected body weight and relative condition factor (Mean±SD) of *Silurus asotus* cultured in captivity for 19 months period

Populations	*W (Observed body weight, g)*	*W' (Expected body weight, g)*	*Kn (Relative condition factor)*
Male	283.84±130.87	241.16±90.31	1.17±0.14
Female	448.14±157.30	446.71±132.82	1.00±0.09
Both sexes	341.76±154.67	338.99±140.35	1.01±0.11

Table 4

The observed, expected body weight and relative condition factor (Mean±SD) of *Silurus asotus* cultured in captivity for a 48 months period

Populations	*W (Observed body weight, g)*	*W' (Expected body weight, g)*	*Kn (Relative condition factor)*
Male	504.01±179.99	497.18±153.05	1.01±0.19
Female	682.01±245.33	666.04±188.36	1.03±0.40
Both sexes	597.09±233.58	583.78±188.93	1.03±0.43

Reproductive performance. The reproductive performance of *S. asotus* cultured in captivity for 19 and 48 months were compared using a Student's t-test with a significance level at p=0.05, as presented in Table 5. For a 19 months period, there were significant differences in the gonadal weight, between sexes: (3.14±1.05 g in males and 19.96±14.54 g in females; t=-2.6; p=0.03). Similar findings were observed in the GSI (1.66±0.32 for males and 8.342±4.26 for females; t=-3.5; p=0.008). Meanwhile, for 48 months, there were also significant differences in the gonadal size, between sexes (3.90±1.23 for males and 18.4±5.34 for females; t=-2.6; p=0.03). In contrast, the GSI index showed no significant difference between male, 1.66±0.32, and female, (8.342±4.26) (t=-3.5; p=0.12, since the p>0.05).

Table 5

The comparison of reproductive performances (Mean±SD) of male and female *Silurus asotus* culture in captivity for periods of 19 and 48 months using a Student's t-test

Months	*Parameter*	*Sex*		*T test*	
		Male (M±S.D.)	*Female (M±S.D.)*	*t*	*p value*
19	Gonad (g)	3.14±1.05	19.96±14.54	-2.6	0.03
	GSI (%)	1.66±0.32	8.342±4.26	-3.5	0.008
48	Gonad (g)	3.90±1.23	18.4±5.34	-5.12	<0.001
	GSI (%)	2.20±0.70	4.18±2.45	-1.74	0.12

GSI-Gonadosomatic index; M-Mean; S.D.-Standard deviation; t-t test; p value-significant p<0.05.

Morphology and endoscopic assessment. Observation of external morphology is shown in Figure 4 and Figure 5 and represents *S. asotus* cultured for 19 and 48 months. There were no differences in morphological characteristics between the male and female (Figure 4: a and b), including the tail part (Figure 5: a and b) for 19 months. However, it was clearly observed that the male *S. asotus* cultured for 48 months had a split tail compared to the female with a non-split tail (Figure 5: c and d). Based on the sex determination results, it was assumed that the split tail was found only in male, while the non-split tail was found only in female (Table 6). During endoscopic surgery, the testes were observed and the milky color of the testes with branched veins was noted (Figure 6). Figure 7 shows the pigmented ovary with a typical cluster "green grape" shape, which

was observed in all fish with unsplit caudal fin. The results were confirmed by the endoscopic intervention, with the fiberscope and the otoscope.

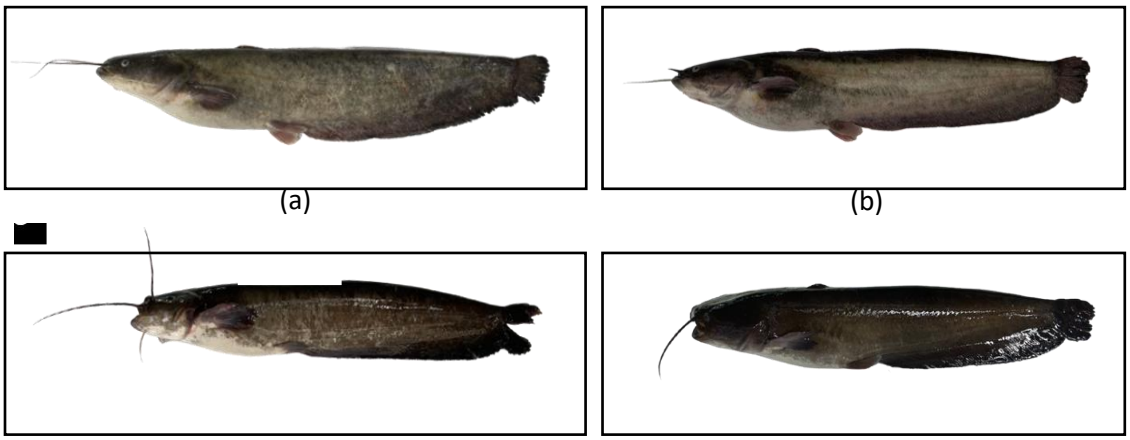

Figure 4. Comparison of external morphology of *Silurus asotus*, (a) male and (b) female (19 months period); (c) male and (d) female (48 months period).

Table 6

The percentage of male and female that have split caudal fin and non-split caudal fin in 48 months culture period

Sex	*N*	*Caudal fin*	*Percentage (%)*
Male	10	Split	100
Female	10	Non Split	100

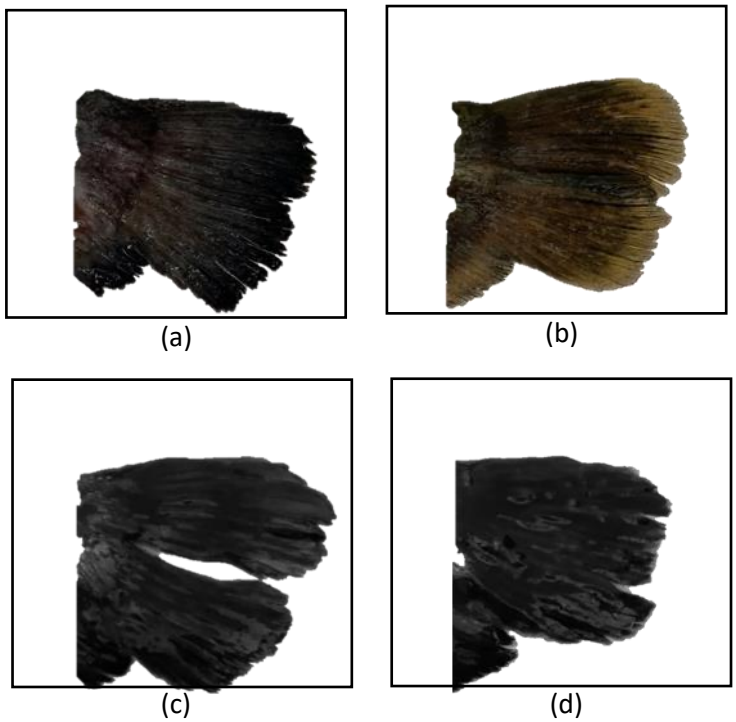

Figure 5. Comparison of tail part for female and male of *Silurus asotus*, (a) male and (b) female (19 months period); (c) male and (d) female (48 months period).

Figure 6. Milky color testes with arborizing vein detected in all males with split caudal fin.

Figure 7. The pigmented ovary's typical cluster of "green grape" shape, detected in all female with non-split caudal fin.

Discussion

Length-weight relationship (LWR). The LWR of *S. asotus* cultured for 19 months showed that both sexes achieved a positive allometric growth, as the b value was 3.04 (>3), while the male and female populations independently achieved a negative allometric growth, as the b value was 2.95 (<3) and 2.91 (<3), respectively. However, for a 95% confidence interval, all populations cultured during a 19 months period were in the range of 3.0, indicating that both the female and male populations achieved the ideal growth. The opposite results were found for a 48months culture period. The b-value of the male (2.91), female (2.31) and both sexes (2.66) all exhibited a negative allometric growth. This indicates that the fish species exhibits a relatively slow growth. In fact,

younger specimens tend to show larger b-values than adult specimens in each population and fish species (Froese 2006). The LWR of 313 *C. gariepinus* was examined using a similar approach and the estimated b values for male, female and both sexes were 7.74, 6.96 and 7.87, respectively (De Giosa et al 2014). The differences in b-values between the previous and the present study may be attributed to the small size of the fish studied (TL 22.1-28.5 cm) (De Giosa et al 2014). This result may also be influenced by one or a combination of factors, including the number of specimens sampled, sex, area and seasonal effects, as well as by differences in the observed length ranges of specimens captured and by the duration of sampling (Moutopoulos et al 2002). It has been observed that the growth of the *S. asotus* is influenced by a mechanism that channels energy for sexual maturation rather than promoting growth (Sariat et al 2020). The t-test showed significant differences, $p<0.05$ in growth performance between males and females, in both culture periods, as shown in Table 3. The present study also showed that females can grow faster than males in both experimental groups, within the 19 and 48 months of culture periods. This finding indicates that the female is larger than the male in each production population. This is consistent with previous studies showing that female are larger than male due to the genetic basis of sexual dimorphism in *S. asotus* (Shen et al 2020). On the other hand, male of several catfish species, such as blue catfish (*Ictalurus furcatus*), yellow catfish (*Pelteobagrus fulvidraco*) and flathead catfish (*Pylodictus olivaris*), grew faster and reached a larger size than the female (Lu et al 2015; Esmaeili et al 2017; Shen et al 2020). However, in the *C. gariepinus* study (Shola et al 2015), there were no size differences between adult males and females. The result of the present study is consistent with previous studies, reporting the excellent growth rate of *S. asotus*, which is equally applicable to other aquaculture fish species (Yang et al 2015; Sariat el al 2020).

Relative condition factor (RCF). It is known that the condition of the fish body varies depending on the changes in gonadal development, food availability and other environmental factors (Yang et al 2015). The RCF of the *S. asotus* cultured in captivity for 19 and 48 months are provided in Table 4 and Table 5. In this study, although a negative allometric growth was observed in the LWR results, *S. asotus* was larger than 1.0 (1.00-1.17) for each culture period in the overall population. However, no study has been done to evaluate *S. asotus* broodstock RCF in captivity. According to a study (Keyombe et al 2015) on RCF of the *C. gariepinus* males, females and both sexes, values of 0.5771, 0.5284 and 0.5527, respectively, were determined in wild culture, which indicate an unhealthy status of the population. This may be due to a reduced availability of food and prey populations in the wild environment. The *S. asotus* specimens in this study were found to be healthy, robust and in a good condition compared to the *C. gariepinus* in the wild. This finding proved that intensive broodstock management, in particular feeding management and water quality monitoring, is essential to the *S. asotus* performance in captivity.

Reproductive performance. The reproductive performance of *S. asotus* cultured in captivity for 19 and 48 months was compared using Student's t-test with significant values at $p<0.05$, as shown in Table 5. At 19 months, there were significant differences in gonad between sexes ($p=0.03$), with the gonad of the female being larger than that of the male. There were also significant differences in the GSI values ($p=0.008$). Thus, the GSI of *S. asotus* was higher in the female than in the male specimens. This means that the body weight of the fish is inversely proportional to its gonad weight. Female have a higher gonadal weight due to the presence of eggs in the enlarged ovaries when they reach their mature stage (Pope et al 1996). The low value of GSI in male is due to the very low energy expenditure during the gametes production. In contrast, *S. asotus* showed significant differences in gonads between sexes during the 48 months culture period ($p=0.03$). However, there were no significant differences in GSI between the male and female ($p=0.12$). This finding suggests that the development of the gonad was in the spent stage, i.e., the fish were not yet in their peak season. As suggested in previous studies, the GSI values follow the gonadal maturation, reaching higher levels at the

mature stage, then decreasing after spawning and gametogenesis, especially in the female species (Pope et al 1996).

Morphology and endoscopic assessments. Sexes determination by genital papillae has been used in many animal species, especially catfish. Studies have shown that other external morphological characteristics, such as body coloration, body size and the formation of a hump on the head are indicators of sex determination in some species (Ramasamy 2019; Tagarao et al 2020). The shape of the genital papillae, which are almost identical in *S. asotus*, has led to difficulties and errors in broodstock selection. As for the Bagrid catfish (*M. pelusius* Solander, 1794), the male has small, fleshy, pointed genital papillae. In addition, the pelvic fin was shorter and did not extend back to the genital papillae (Esmaeili et al 2017). In female *M. nemurus* the opening of the genital structure was without any projection and the pelvic fin extended to and overlapped the genital papillae (Esmaeili et al 2017). Apart from the shape of the genital papillae, the color of the female Southern catfish (*Glyptothorax silviae*) was darker than that of the male species (Esmaeili et al 2017). However, the present study showed that the tail of male *S. asotus* started to split when they were cultured for 48 months, compared to the group that was cultured for only 19 months. The identification method based on morphological characteristics contributed to a better management of the broodstock selection of *S. asotus*.

Conclusions. The study concluded that *S. asotus* are able to attain an excellent growth in captivity and display a good health under proper broodstock management, between 19 to 48 months of culture. Although *S. asotus* are generally grown under temperate environments, they can show a better growth under controlled conditions, in tropical environments as well. This study also revealed a method of sex differentiation in matured *S. asotus*, based on the presence of split caudal fin only in male specimens. This sex identification is unique to *S. asotus* and it is practical and easy to use by farmers, instead of the conventional method of sacrificing fish. *S. asotus* is a new species in the aquaculture research, particularly in tropical regions. More studies should be done in order to enhance the quality of broodstock, especially on the nutrient requirements to improve maturation and egg quality. The new endoscopic technique proposed in this study is also recommended to be applied on other species.

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Conflict of interest. The authors declare no conflict of interest.

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Authors:
Hanis Abd Mohmin, Malaysia Sabah University, Borneo Marine Research Institute, Kota Kinabalu 88540, Sabah, Malaysia; Kindai University, UMS-Kindai Aquaculture Development Centre, 1-5 Shirahama, Wakayama, 649-2211, Japan, e-mail: hanismohmin2@gmail.com
Norfazreena Mohd Faudzi, Malaysia Sabah University, Borneo Marine Research Institute, Kota Kinabalu 88540, Sabah, Malaysia, e-mail: fazreenafaudzi@ums.edu.my
Fui Fui Ching, Malaysia Sabah University, Borneo Marine Research Institute, Kota Kinabalu 88540, Sabah, Malaysia, e-mail: cfuifui@ums.edu.my
Shigeharu Senoo, Malaysia Sabah University, Borneo Marine Research Institute, Kota Kinabalu 88540, Sabah, Malaysia; Kindai University, UMS-Kindai Aquaculture Development Centre, 1-5 Shirahama, Wakayama, 649-2211, Japan, e-mail: sesige@mac.com

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