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Sexual characteristic differences between male and female of jade perch *Scortum barcoo*

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Abstract

This study was performed in order to identify sexual differentiation characters of Jade perch as farmers have claimed to have difficulty to identify male and female for reproduction purpose. In order to address this issue, the morphometric characters differences between female and male was studied. Gonadal histological analysis were further applied to confirm sexual identification characters. In the present study, correlations and linear regression analysis were performed for selected 29 morphometric and 11 meristic characters in relation to total length (TL) on 50 specimens. Significant sexual dimorphism between male and female ($p < 0.05$) found in six morphometric characters differences as body depth; head depth; anal fin length; Posterior end of the Dorsal fin to Origin of the Anal fin; Origin of the Dorsal fin to Insertion of the Pelvic fin; and pre-orbital length. Morphometrical analysis revealed that female have slightly larger body width, while the male have a bit longer anal fin and more tapered head. Gonadal histology used for confirmation the distinguish between the male and female, which elucidated different developmental stages in of testis and ovary both. Thus, our present study proved that meristic characters cannot be used to differentiate sexes. Nevertheless, morphological characters can be identified phenotypic variations between the male and female in accompanied with gonadal histology, and the shape of genital papilla.

Keywords: aquaculture, gonad development, morphology, meristic, sex different

1. Introduction

Morphological measurement is of the main research approaches use in ichthyology and aquaculture practice to distinguish species and sexual differences of fishes [1, 2, 3, 4] most likely due to its conventional, simplicity, and precision [5, 6]. Morphometric and meristic characteristics are potential and valuable morphological measurement used in accurate identification of fish species [7, 8], it's tools used to differentiate closely related species of organism having huge similarity indices of various parameters [9]. In principle, morphometric assessment is use to reveal interrelationship among bodily parameters like length, weight, sexual, fecundity, condition factor and many other in understand fitness performance of fishes at different ages [10, 11, 12]. Several studies of descriptive morphology focused on sexual growth dimorphism [13]. Identification of fish sex is critical step to breed in captivity [14, 15], failed to differentiate the sex of fish mean a collapse in the whole coming steps of fish culture. Additionally, histology examination is another approach to confirm sexual differences and gonadal development status for species with similarity characters between male and female of fishes for aquaculture purposes [16, 17].

Jade perch, *Scortum barcoo* is a species potentially for aquaculture farming with it high nutritional value for human consumption and market price. However, current jade perch farming facing seed supply limitation due to inconsistent and difficulty in seed production. One of the seed production difficulty is sexual identification of jade perch. With smaller size of genital papillae, cannulation examination is not suitable to be used and easily cause injury to female fish. Moreover, male fish of jade perch is difficult to strip to examine milky milt availability. Jade perch stomach contains about 60% visceral fat which increase difficulty of stripping method.

Therefore, this study was designed with objective to examine morphological differences between male and female of jade perch. In further, histological analysis was performed to confirm sexual differences by inspecting gonad stage. With confirmation morphology characteristic differences between male and female allow farmer to induce jade perch breeding

artificially with hormonal administration at maturation age. Thus, increase hatchery seed production supply for jade perch farming.

2. Materials and Methods

2.1 Specimen and management

A total 50 of three years old jade perch were obtained from Biodynamic Aquaculture Technology Sdn. Bhd. farm and transferred back to recirculating aquaculture system at Institute of Tropical Aquaculture hatchery, Universiti Malaysia Terengganu. All fishes were distributed randomly in five 500L rectangular fiberglass tanks equipped with external

biological filtration system. All fishes were fed twice a day at 4% of body weight with partial water replacement at 30% every two weeks.

2.2 Morphometric and meristic examination

Randomly, jade perch were sampled from culture system for morphologically analysis and gonadal examination. Selected specimen were anaesthetized with neutralized overdose MS222 prior measurement. Total of 40 morphological characters were selected with 29 morphometric characters (*M*) and 11 meristic characters (*m*) (Table 1 & Fig. 1).

Table 1: Morphometric and meristic characters measurements and definition.

No	Characters	Acronyms
(I) Morphometric characters		
1.	Body Weight	BW
2.	Total Length	TL
3.	Standard Length	SL
4.	Fork Length	FL
5.	Body Depth	BD
6.	Length of the Head	HD
7.	Head Depth	HD
8.	Snout Length	snL
9.	Base length of Dorsal Fin	BDL
10.	Posterior end of the Dorsal fin to Dorsal origin of the Caudal fin	PDDC
11.	Dorsal origin of the Caudal fin to Ventral origin of the Caudal fin	DCVC
12.	Ventral origin of the Caudal fin to Insertion of the Anal fin	VCIA
13.	Length of the Anal fin	LA
14.	Base length of the Anal fin	BA
15.	Origin of the Anal fin to Insertion of the Pelvic fin	OAIP
16.	Length of the Pelvic fin	LP
17.	Posterior end of the Dorsal fin to Insertion of the Anal fin	PDIA
18.	Posterior end of the Dorsal fin to Origin of the Anal fin	PDOA
19.	Origin of the Dorsal fin to Insertion of the Pelvic fin	ODIP
20.	Caudal peduncle Length	CL
21.	Caudal peduncle Depth	CD
22.	Pre-dorsal Length	PrDL
23.	Pectoral fin Length	PcFL
24.	Upper jaw length (pre-orbital length)	PrOL
25.	Eye diameter	ED
26.	Caudal height	CH
27.	Pre-pectoral length	PPL
28.	Pre-pelvic length	PVD
29.	Pre-anal length	PAL
(II) Meristic characters		
A.	Pectoral fin rays	PFR
B.	Ventral fin spines	VFS
C.	Ventral fin rays	VFR
D.	Anal fin spines	AFS
E.	Anal fin rays	AFR
F.	Dorsal fin spines	DFS
G.	Dorsal fin rays	DFR
H.	Caudal fin rays	CFR
I.	Lateral line scales	LLS
J.	Scale row above lateral line	SrALL
K.	Pre-dorsal scales	PDS

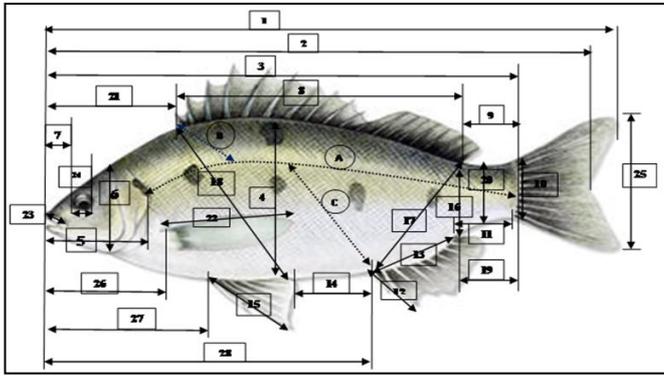


Fig 1: Diagram of morphometric and meristic characteristic measurements of *S. barcoo*.

2.3 Histological gonadal examination

After morphometric measurement, fish were dissected and gonad were removed for sex identification. After weighing, the gonad samples were fixed in Bouin’s solution for 24 hours followed by dehydration steps with ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Samples were sectioned at 5 µm and stained with Haematoxylin and Eosin, and mounted on a glass slide with DPX mounting medium. Slides were then viewed with advanced compound microscope (Nikon Eclipse 80i) at x7 and x10 magnification, linked to computer image analysis software (Olympus Micro Suite™ FIVE) to determine the sex of the specimen.

2.4 Statistical analysis

Correlation analysis was performed to examine morphometric characters relationship with body length for both male and female. To examine the morphometric sexual differences of

jade perch, each measurement characters were compared by using unpaired Student T-test. All data were checked for normality distribution, outlier data were removed from analysis. Significant level was set at 95% confident limit (P<0.05).

3. Results and Discussions

Correlation coefficients (r) of morphometric traits in both sexes showed a highly significant correlation (P<0.01) in most variables. The lowest correlation coefficient was between total length (TL) and eye diameter (ED) for female (r = 0.061), and total length (TL) and Posterior end of the Dorsal fin to Dorsal origin of the Caudal fin (PDDC) for male (r =0.042). The highest correlation coefficient (r) was observed between total length (TL) and Fork length (FL) in both female and male estimated at 0.989 and 0.976, respectively (Table 2 and 3).

From 28 morphometric characters, results showed that 26 characters in female fish (p<0.01) and 24 characters in male fish (p<0.05) were positive correlated significantly. Two characters in female fish were found not significant which were ED and CH. While, 4 characters included PDDC, LP, CL, and CH were also found not significant in male fish (Table 2 and 3).

Table 4 showed the comparative morphometric characters between female and male of jade perch. Overall comparison found that 6 morphometric characters were significantly different which were BD, HD, AFL, PDOA, ODIP, and PrOL. Measurements showed that HD, AFL, PrOL in male were significantly higher than in female fish. On the other hand, BD, PDOA and ODIP characters were higher in female than male fish.

Table 2: Relationship between body lengths with other morphology characters of females jade perch *S. barcoo*. Double asterisk (**) indicated highly significant with p<0.01.

Morphometric characters	r	r ²	increment	correlation	t-stat	Significance (p-level)
SL	0.962	0.888	0.940 (-A)	0.962**	15.743	2.5E-016
BW	0.843	0.747	0.013(-A)	0.843**	9.570	9.1E-11
FL	0.989	0.935	0.916(-A)	0.989**	21.190	5.3E-20
BD	0.920	0.517	1.606(+A)	0.920**	5.759	2.4E-06
HL	0.861	0.741	2.333(+A)	0.861**	6.095	3.8E-05
HD	0.566	0.321	1.577(+A)	0.566*	2.476	0.028
SnL	0.814	0.662	5.820(+A)	0.814**	5.043	0.000
DFL	0.874	0.764	1.234 (+A)	0.874**	6.492	2E-05
PDDC	0.726	0.526	2.645(+A)	0.726**	3.800	0.002
DCVC	0.730	0.533	3.831(+A)	0.730**	3.848	0.002
VCIA	0.647	0.419	2.891(+A)	0.647**	3.059	0.009
AFL	0.791	0.625	4.804(+A)	0.791**	4.655	0.000
BA	0.811	0.657	3.701(+A)	0.811**	4.989	0.000
OAIP	0.821	0.675	1.774(+A)	0.821**	5.191	0.000
LP	0.544	0.296	3.938(+A)	0.544*	2.338	0.036
PDIA	0.876	0.767	3.314(+A)	0.876**	6.540	1.9E-05
PDOA	0.913	0.834	2.275(+A)	0.913**	8.090	2E-06
ODIP	0.782	0.611	1.420(+A)	0.782**	4.518	0.021
CL	0.647	0.418	2.629(+A)	0.647**	3.057	0.009
CD	0.812	0.659	5.064(+A)	0.812**	5.007	0.000
PrDL	0.908	0.824	2.632(+A)	0.908**	7.809	2.9E-06
PcFL	0.719	0.517	4.457(+A)	0.719**	3.733	0.003
PrOL	0.633	0.400	5.599(+A)	0.633*	2.945	0.011
ED	0.061	0.004	3.115(+A)	0.061	0.221	0.828
CH	0.252	0.063	0.672(-A)	0.252	0.938	0.365
PPL	0.780	0.609	5.070(+A)	0.780**	4.499	0.001
PVD	0.531	0.282	2.301(+A)	0.531*	2.258	0.042
PAL	0.951	0.904	1.440(+A)	0.951**	11.046	5.6E-08

Table 3: Relationship between body lengths with other morphology characters of males jade perch *S. barcoo*. Double asterisk (**) indicated highly significant with $p < 0.01$.

Morphometric characters	r	r ²	increment	Correlation	t-stat	Significance (p-level)
SL	0.943	0.788	0.866(-A)	0.943**	7.475	2E-06
BW	0.914	0.868	0.014(-A)	0.914**	9.949	5.3E-08
FL	0.976	0.915	0.991(-A)	0.976**	12.673	2E-09
BD	0.744	0.825	1.665(+A)	0.744**	8.396	4.7E-07
HL	0.846	0.716	2.476(+A)	0.846**	5.494	0.000
HD	0.713	0.509	1.172(+A)	0.713**	3.526	0.004
SnL	0.696	0.485	7.328(+A)	0.696**	3.361	0.006
DFL	0.937	0.878	1.285(+A)	0.937**	9.308	7.7E-07
PDDC	0.042	0.002	0.095(-A)	0.042	0.146	0.887
DCVC	0.777	0.603	5.476(+A)	0.777**	4.273	0.001
VCIA	0.800	0.640	2.217(+A)	0.800**	4.623	0.001
AFL	0.917	0.840	3.574(+A)	0.917**	7.951	4E-06
BA	0.829	0.687	4.810(+A)	0.829**	5.131	0.000
OAIP	0.762	0.580	1.795(+A)	0.762**	4.072	0.002
LP	0.244	0.059	1.102(+A)	0.244	0.870	0.401
PDIA	0.746	0.556	3.744(+A)	0.746**	3.875	0.002
PDOA	0.876	0.767	2.999(+A)	0.876**	6.291	4E-05
ODIP	0.689	0.474	1.707(+A)	0.689**	3.290	0.006
CL	0.365	0.133	1.107(+A)	0.365	1.357	0.200
CD	0.817	0.667	7.614(+A)	0.817**	4.905	0.000
PrDL	0.793	0.629	2.407(+A)	0.793**	4.509	0.001
PcFL	0.693	0.481	5.441(+A)	0.693**	3.333	0.006
PrOL	0.722	0.521	9.458(+A)	0.722**	3.612	0.004
ED	0.557	0.310	21.150(+A)	0.557*	2.322	0.039
CH	0.476	0.226	2.099(+A)	0.476	1.873	0.086
PPL	0.826	0.683	3.428(+A)	0.826**	5.083	0.000
PVD	0.765	0.585	2.047(+A)	0.765**	4.114	0.001
PAL	0.929	0.863	1.571(+A)	0.929**	8.681	1.6E-06

Table 4: Mean of comparative morphometric measurements of between female and male jade perch *S. barcoo*. Significant ($p < 0.05$) is indicated with (S) and not significant (NS).

Characters	Female	Male	T-test	Sig. Diff ($p < 0.05$)
	Mean±SE	Mean± SE		
TL	29.12±0.33	29.3±0.44	-0.338	0.74 (NS)
SL	25.46±0.33	25.99±0.45	-0.957	0.345 (NS)
BW	621.36±21.83	668.55±29.90	-1.275	0.211 (NS)
FL	27.93±0.35	28.06±0.42	-0.242	0.810 (NS)
BD	10.60±0.15	11.34±0.24	-2.636	0.014 (S)
HL	5.73±0.17	6.04±0.16	-1.283	0.210 (NS)
HD	4.99±0.17	5.73±0.29	-2.202	0.039 (S)
SnL	1.89±0.06	1.96±0.05	-0.984	0.336 (NS)
DFL	14.43±0.33	15.05±0.35	-1.306	0.203(NS)
PDDC	3.55±0.13	3.44±0.21	0.424	0.676 (NS)
DCVC	3.71±0.09	3.69±0.07	0.124	0.902 (NS)
VCIA	3.77±0.10	4.03±0.17	-1.273	0.216 (NS)
AFL	4.04±0.08	4.34±0.12	-2.056	0.051 (S)
BA	3.77±0.10	4.02±0.08	-1.955	0.061 (NS)
OAIP	9.68±0.21	9.6±0.20	0.272	0.788 (NS)
LP	4.15±0.06	4.35±0.11	-1.598	0.124 (NS)
PDIA	4.24±0.12	4.46±0.09	-1.404	0.172 (NS)
PDOA	7.29±0.19	7.91±0.14	-2.706	0.012 (S)
ODIP	9.14±0.25	9.89±0.19	-2.362	0.026 (S)
CL	4.81±0.11	5.04±0.16	-1.148	0.262 (NS)
CD	3.17±0.07	3.34±0.05	-1.958	0.062 (NS)
PrDL	8.28±0.16	8.55±0.16	-1.207	0.238 (NS)
PcFL	4.07±0.07	4.09±0.06	-0.203	0.841 (NS)
PrOL	1.33±0.05	1.5±0.04	-2.619	0.015 (S)
ED	1.01±0.01	1.03±0.01	-0.985	0.335(NS)
CH	6.99±0.17	6.89±0.11	0.527	0.603(NS)
PPL	5.68±0.07	5.85±0.11	-1.259	0.221 (NS)
PVD	8.09±0.11	8.39±0.18	-1.410	0.173 (NS)
PAL	17.35±0.31	18.06±0.28	-1.695	0.102 (NS)

Present study revealed that a total of 29 morphometric and 11 meristic characters between male and female jade perch shown no obvious differences phenotypically, except 6 characters. Similarity morphometric between male and female character is a common sign among fishes, not only perch family. The growth differences among the body parts in relation with the total length at different life stages did not exhibit isometrically [18]. Differently, myriad species exhibited sexually dimorphic growth pattern differently with one sex reaches a larger ultimate size than the other [19, 20]. The differences of BD, HL, AFL, PDOA, ODIP and PrOL characters between male and female of jade perch is believed in associated with their maturation stages. Although, not obvious as compared to others species with significant extended long fins and colour change [21, 22, 23]. Meristic counts analysis for both sexes revealed similarity, and not different significantly at $p > 0.05$. May be due to what stated by [24, 25] meristic characters were independent of size of the fish and did not change during growth. Many authors considered meristic characters less useful when comparing morphological variations [26].

In addition, genital papillae structure between male and female shown different structure basically at mature age, agreed to what mentioned by [27, 28]. Female fish have larger and rounded shape with difference of 2 curve were notice. While for male fish have slightly small with tip structure and generally contains 4-5 curves as shown in Figure 2.



Fig 2: The differences the genital papilla structure between male and female of jade perch

This confirmation was proven with gonadal histological examination. Histologically, several different stages of spermatogenic cysts were observed such as spermatogonia, spermatocytes, spermatids, and spermatozoa within the lamellae of the testis (Figure 3. a-d). Mainly, in testis of immature stage presence small spermatogenic, lobule lumina and duct networks even poorly developed, and closely packed primary growth stage (chromatin nucleolar, peri-nucleolar oocytes). Also Spermatocytes was recognizable with the small nuclear with chromatin cells (Figure 3.a) Spermatogenic cysts contained spermatogonia, spermatocytes both primary and secondary, and spermatids were also noticed (Figure 3.b and 3.c). A single spermatogenic cyst contained dominant spermatozoa was noticed (Figure 3.d).

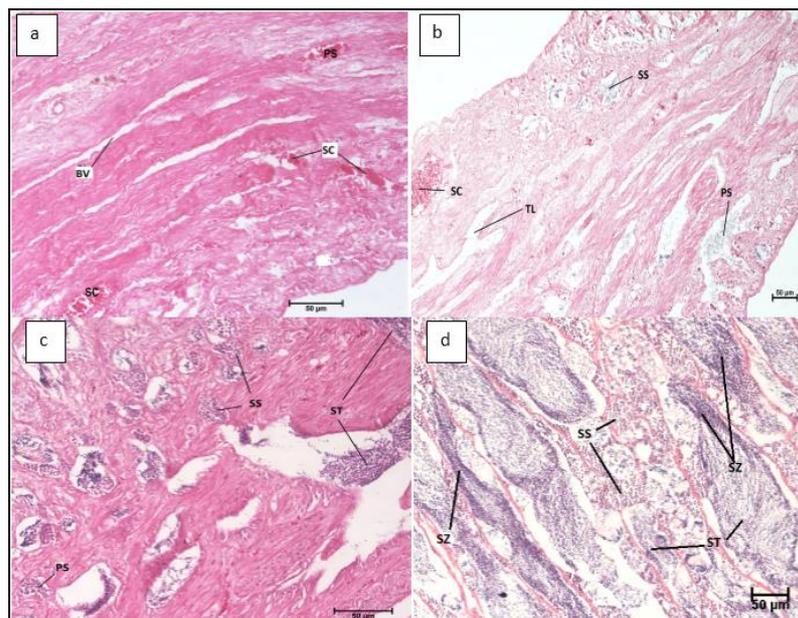


Fig 3: Histological sections of male *S. barcoo* gonads. (a) Immature (b) developing male; (c) maturing male; (d) mature male, 50 µm Scale bar. SG, spermatogonia; SC, spermatogonia cyst; BV, blood vessel; TL, tubule lumen; PS, primary spermatocytes; SS, secondary spermatocytes; ST, spermatid; SZ, spermatozoa.

For female, ovary was dominated by the presence of vacuolated oocytes and difference sizes of oocyte with cytoplasm basophilic. Immature cells with small vacuoles were observed at the periphery of the oocyte and indicated the vacuoles gradually move towards the nucleus of mature cells (Figure 4. a-d). Ovary is undergoes a series of developmental stages, where multiple nucleoli in the nucleus of the (germinal vesicle) oocytes were observed in immature stage (Figure 4.a). Then granular structures increased with the ooplasm, cortical alveoli proliferate and follicle development as oocyte

developed and became opaque in the area that surrounded the nucleus. Thereby, nucleoli cells were pushed from the nuclear envelope and zona radiata (Vitelline envelope) begin to form and the follicle epithelium became thicker (Figure 4.b). Cortical vesicles were in spherically shape on the periphery of the cytoplasm at vitellogenic stage (Figure 4.c). Followed by vitelline membrane constituted the inner zone of the vitelline envelope started to disintegrate by leaving the void spaces from the outer parts (Figure 4.d).

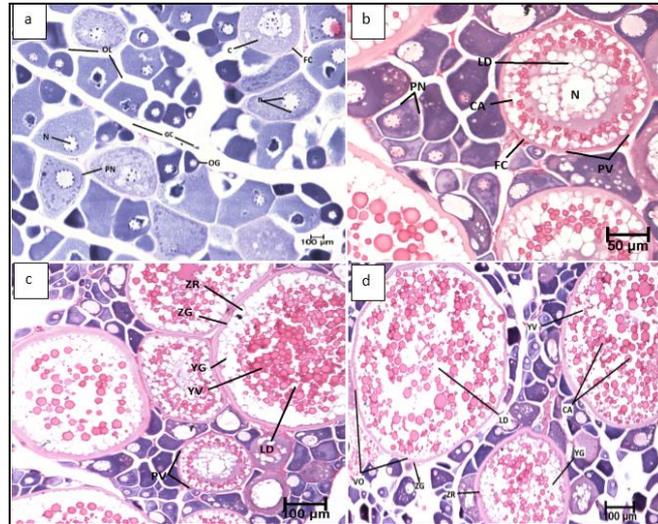


Fig 4: Histological sections of female *S. barcoo* gonads. (a) Immature, 100 µm (b) developing female, 50 µm (c) maturing female, 100 µm (d); matured female 100 µm. OG, oogonia; C, cytoplasm; FC, follicle cell; GC, germinal cell; OL, ovarian lamelle; PN, perinucleolus oocytes; PV, previtellogenic oocytes; VO, vitellogenic oocytes; OC, ovarian cavity; LD, lipid droplets; N, nucleus; n, nucleolus; CA, cortical alveoli; YG, yolk granules; YV, yolk vesicles; ZG, zona granula; ZR, zona radiate.

The histological data of gonadal classes provided evidence of the sexual pattern and developmental pathways of *S. barcoo*. The finding indicated that gonadal development passed through different phases and stages. In which, oogonia transformed into primary oocytes [29, 30], chromatin nucleolus and subsequent perinucleolus stages are found in both immature and mature fish [29], primary oocyte (chromatin nucleolar stage) contained a single large, centrally located nucleus surrounded by a thin layer of cytoplasm. Externally it is enclosed by the initial follicle, which consist of a few squamous follicle cells [31, 32]. In the early perinucleolus stage the nucleus increased in size and multiple nucleoli formed in the peripheral margin [33]. Lipid droplets begun to accumulate in the cytoplasm at about the same time as the formation of cortical alveoli precursors [31, 32]. Vitellogenesis identified lipid droplets prior to the accumulation of yolk globules, and then granular yolk [34].

4. Conclusion

As a conclusion, jade perch is able to distinguish between male and female morphologically by examined the genital papillae structure and six dimorphic characters (BD, HD, AFL, PDOA, ODIP, and PrOL) at mature age of 3 years.

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