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ORIGINAL ARTICLE

Sex Determination in *Oxyeleotris marmorata* (Bleeker, 1852) Based on Morphometric Features

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Hasan Basri Idris, Mohd Azmi Ambak, Ikhwanuddin Abdullah Kutty; Sex Determination in *Oxyeleotris marmorata* (Bleeker, 1852) Based on Morphometric Features.

ABTRACT

This study was carried out to determine the morphometric and meristic characters of Marble Goby (Oxyeleotris marmorata) in order to identify possible sexual differentiation characters in this potential aquaculture species. The ratios of urogenital length, pelvic fin length, caudal fin length, pre dorsal length, pre anal length, pre pelvic length, pre pectoral length, caudal peduncle length, body depth, head length, and snout length to the standard length was measured. The only meristic character used in this study was scales on lateral line. The analyses were done in thirty (30) specimens of marble goby. Analysis by using Student 't' test showed three morphometric features have significant values in sexual determination of marble goby namely urogenital length, caudal fin length and caudal peduncle length.

Key words:

Introduction

Most of the fishes exhibit sexual dimorphism or secondary sexual characters by which sexes can be differentiated from each other. In a few fishes secondary sexual characters are discernible throughout the life span where as in some others they are apparent only during the breeding season. Secondary sexual characters serve several function such as recognition of opposite sex by the members of a given sex, helping in the act of copulation such as sexual embrace, transfer of spermatozoa from male to female and facilitating parental care and plays significant role in seed production programme. External morphological differences between male and female are diverse and highly specific from one species to another. Some of them can be distinguished by size of the fish; length, shape and texture of the fin; colouration; genital papilla, presence of organ ovipositor and shape of head.

Secondary sexual characteristic is essential in broodstock management programme in order to develop viable and sustainable aquaculture production. One of the problems appears in mega scale production of marble goby highlighted by local farmer is difficulty to identify sexually mature broodstock. The aim of this study is to determine secondary sexual characteristics of sexually mature marble goby broodstock by using morphometric analysis. Morphometics is the quantitative analysis of form, a concept that encompasses size and shape. Normally morphometrics analyses are performed on organisms. It is useful in analysing fossil record, the impact of mutants on shape, developmental changes in form, covariance between ecological factors and shape and also for estimating quantitave-genetic parameters of shape.

Methodology:

2.1 Collection of specimen:

In October 2010, 30 breeders were obtained from the fresh market at Kuala Terengganu which was collected from wild environment by local people. They were transported to the Freshwater Hatchery, Faculty of Food Science and Agro technology of Universiti Malaysia Terengganu by road. These breeders were placed immediately in the concrete tank (7' X 13' X 2') for conditioning and preparation for the study purpose. The breeders were fed daily with high protein source such as chopped raw fish (*Rastrelliger kanagurta*), small carps

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and squids (*Loligo sp*). Continuous aeration was given. The ambient water quality parameters such as salinity, temperature, pH and dissolved oxygen (DO) were monitored daily.

2.2. Morphometric and meristic observation:

Sexual identification was carried out in the field by examine the external markings (bars and spots), and number of dorsal spines. The weight and length of each specimen were taken by using a top-loading weighing balance and a meter rule, respectively. Morphometric and meristic parameters were made for each specimen as described by Mohsin and Ambak (1983).

The ratios of urogenital papillae, pelvic fin length, pelvic spine length, caudal fin length, pre dorsal length, pre anal length, pre pelvic length, pre pectoral length, caudal peduncle length, body depth, and head length. The same was measured for the snout length to the head length. The only meristic characteristic counted in this study is scales on the lateral line.

2.3. Gonadal Examination:

After doing morphometric and meristic observations, each specimen was dissected and the gonad located, removed and preserved in 4% formaldehyde. Ovary gravid with eggs indicated female sex of the specimen. However, 5m histological section and of the gonad was made and stained with Bouin's Haematoxylin and Eosin. Then specimens were observed under the microscope to determine the sex of the specimen.

2.4 Statistical analysis:

The mean values of ten morphometric ratios and one morphometric feature enumerated for the 30 specimens of *O. marmorata* observed in this study were statistically compared between identified male and female specimens using Student't' test.

Results:

The observed sex ratio was 1:1 (15 males and female) for the 30 specimens of *O. marmorata* concluded in this study. The ranges and means of weight, and the standard and total length of fish samples collected are shown in Table 1. The smallest and the biggest specimens weighed 180 g and 640 kg both of which were males and measure 35 cm and 24 cm, respectively.

Examination of gonad was used to determine the sex of the specimens. As in most teleosts, the gonads in male and female marble goby are paired, elongated organs, located in the dorsal portion of the body cavity.

There is no striking morphological appearance or meristic feature distinguishing the male from the female after conducting physical examination. All appendages looked alike in shape, feel and location on the body for all specimens.

Table 2 and 3 shows ten morphological ratios of the male and female respectively. A comparison of these ratios by using Students't'- test of significance found significant differences in the observation made for only three of the ten morphological ratios (Table 4). The ratio of urogenital length to the standard length observed for male *O. marmorata* was 1.78 ± 0.37, which was significantly lower than ($p<0.05$) than the 2.64 ± 0.52 observed for the female. In caudal fin length the results showed the male has slightly longer than in female which were 16.69 ± 4.31 and 7.55 ± 2.42 respectively. The same pattern was observed in caudal peduncle length where male (10.76 ± 1.64) was significantly longer than in female (6.54 ± 1.56).

Table 1: Weight and length of male and female samples of *Oxyeleotris marmorata*

Parameter		Male	Female
Weight (kg)	Range	180 – 640	185 – 420
	Mean	0.35	0.278
Total length (cm)	Range	24 – 33.5	27.5 – 32.5
	Mean	30.7083	29.8076
Standard length (cm)	Range	19 – 29	20.5 – 26.5
	Mean	24.2916	23.0833

Table 2: Ratios of morphological characters of sample male *Oxyeleotris marmorata*

Morphological	N	Min	Max	Mean	Std
Urogenital length (%SL)	15	1.05	2.41	1.78	0.37
Pelvic fin length (% SL)	15	8.89	17.24	13.14	2.47
Pelvic spine length (%SL)	15	5.77	15.79	11.50	3.12
Caudal fin length (%SL)	15	10.34	23.08	16.69	4.31
Pre dorsal length (%SL)	15	34.29	54.04	43.96	5.84

Pre anal length (%SL)	15	48.08	82.00	65.42	7.92
Pre pelvic length (%SL)	15	26.92	38.30	33.49	3.27
Pre pectoral length (%SL)	15	29.62	40.43	36.05	3.39
Caudal peduncle length (%SL)	15	7.69	13.79	10.76	1.64
Body depth (%SL)	15	17.31	24.14	20.89	2.07
Head length (%SL)	15	25.00	40.91	35.44	4.11
Snout length (%HL)	15	25.00	33.33	28.34	2.53

*n = Sample size Std = Standard deviation

Table 3: Ratios of morphological characters of sample female *Oxyeleotris marmorata*

Morphological	N	Min	Max	Mean	Std
Urogenital length (%SL)	15	2.64	4.39	3.45	0.52
Pelvic fin length (% SL)	15	11.32	20.93	14.48	2.69
Pelvic spine length (%SL)	15	8.70	16.28	12.63	1.99
Caudal fin length (%SL)	15	7.55	15.38	11.45	2.42
Pre dorsal length (%SL)	15	41.15	54.55	46.39	3.38
Pre anal length (%SL)	15	56.60	77.27	68.55	5.32
Pre pelvic length (%SL)	15	33.33	38.64	36.23	1.44
Pre pectoral length (%SL)	15	30.19	40.91	38.12	2.63
Caudal peduncle length (%SL)	15	6.54	11.82	8.87	1.56
Body depth (%SL)	15	17.39	25.00	20.08	1.93
Head length (%SL)	15	30.19	42.31	37.42	3.08
Snout length (%HL)	15	25.88	40.00	28.98	3.57

*n = sample size Std = Standard deviation

Table 4: Comparison of morphological ratios for samples of male and female

Morphological ratio	Male	Female	Sig. Diff ($p<0.05$)
Urogenital length (% SL)	1.78 ± 0.37	2.64 ± 0.52	s
Pelvic fin length (% SL)	13.14 ± 2.47	11.32 ± 2.69	ns
Pelvic spine length (% SL)	11.50 ± 3.12	8.70 ± 1.99	ns
Caudal fin length (% SL)	16.69 ± 4.31	7.55 ± 2.42	s
Pre dorsal length (% SL)	43.96 ± 5.84	41.15 ± 3.38	ns
Pre anal length (% SL)	65.42 ± 7.92	56.60 ± 5.32	ns
Pre pelvic length (% SL)	33.49 ± 3.27	33.33 ± 1.44	ns
Pre pectoral length (% SL)	36.05 ± 3.39	30.19 ± 2.63	ns
Caudal peduncle length (% SL)	10.76 ± 1.64	6.54 ± 1.56	s
Body depth (% SL)	20.89 ± 2.07	17.39 ± 1.93	ns
Head length (% SL)	35.44 ± 4.11	30.19 ± 3.08	ns
Snout length (% HL)	28.34 ± 2.53	25.88 ± 3.57	ns
Scales on lateral line	80.80 ± 1.26	81.27 ± 1.83	ns

* Mean ± standard deviation (cm) s = significant ns = not significant

Table 5: Definitions of morphometric measurements and meristic counts of marble goby in this study

Character	Description	Acronym
Standard length	Tip of the upper jaw to the tail base	SL
Pelvic fin length	From base to tip of the pelvic fin	PVFL
Pelvic spine length	From base to tip of pelvic spine	PVSL
Caudal fin length	From tail base to tip of the caudal fin	PFL
Pre-dorsal length	Front of the upper lip to the origin of dorsal fin	CFL
Pre-anal length	Front of the upper lip to the origin of anal fin	PAL
Pre-pectoral length	Front of the upper lip to the origin of pectoral fin	PPCL
Snout length	The front of the upper lip to the flesy anterior edge of the orbit	STL
Caudal peduncle length	From base of the last anal fin ray to middle of caudal fin fold	CPL
Body depth	Maximum depth measured from the base of the dorsal spine	BD
Head length	From the front of the upper lip to the posterior end of the opercular membrane	HL

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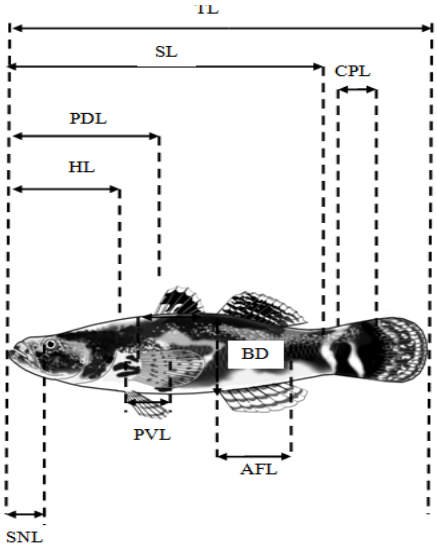

Fig. 1: Morphometric measurements of marble goby

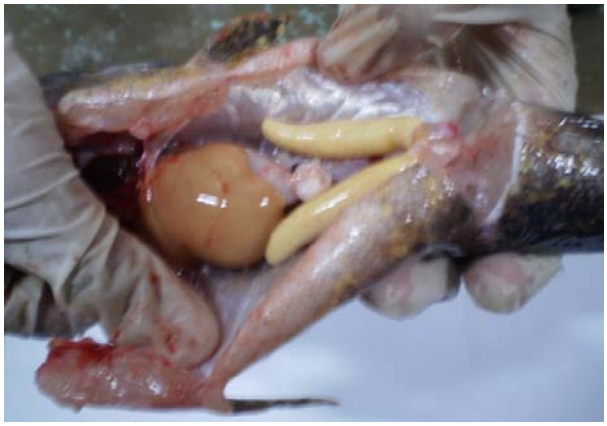

Fig. 2: Female marble goby

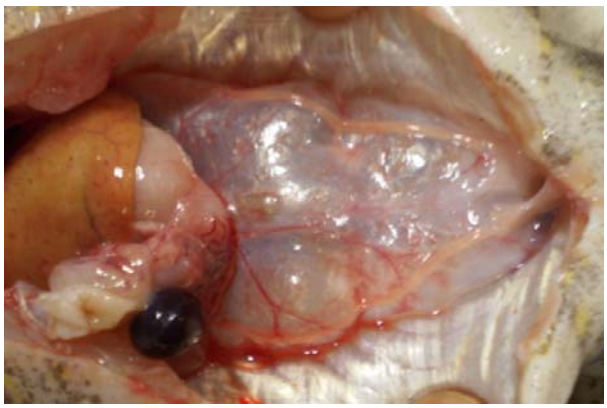

Fig. 3: Male marble goby

Fig. 4: Male and female of marble goby

Fig. 5: Marble goby on a measuring board

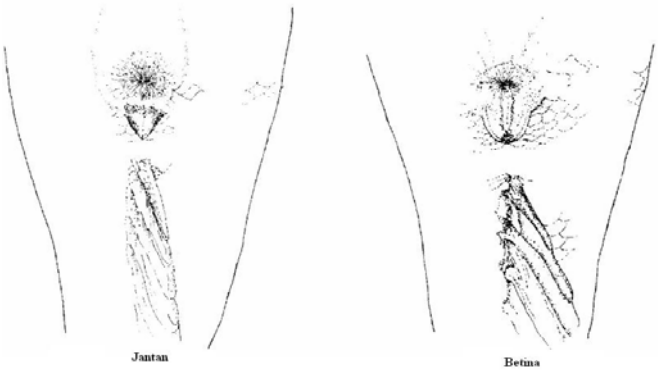

Fig. 6: Morphological difference between male and femaleCourtesy of Marble Goby Farm, Sg. Petani, Kedah

Figure (6-11): Testes of *Oxyeleotris marmorata* at different stages of maturation:

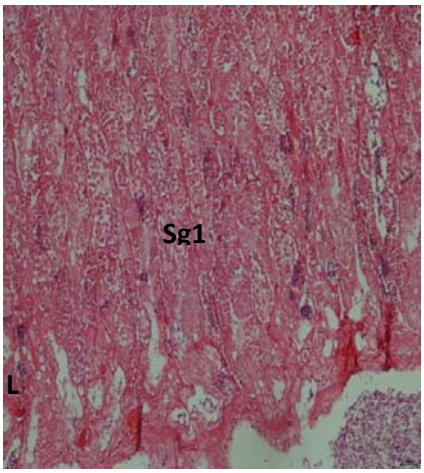

Fig. 6: Immature stage

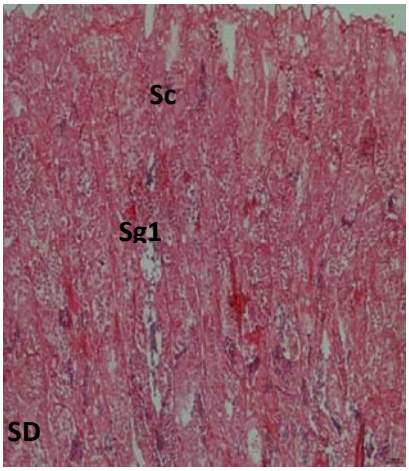

Fig. 7: Immature stage

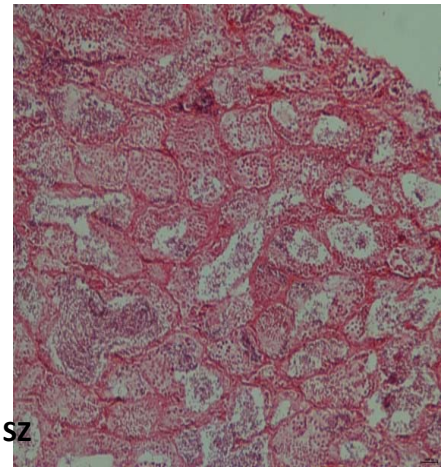

Fig. 8: Developing stage

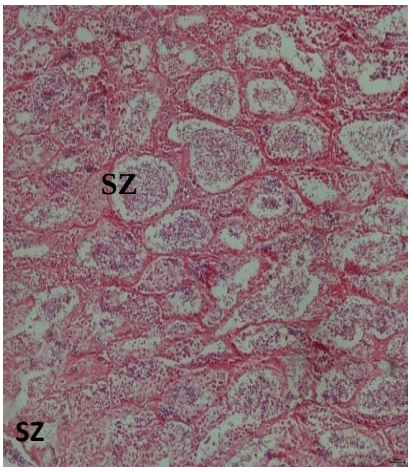

Fig. 9: Developing stage

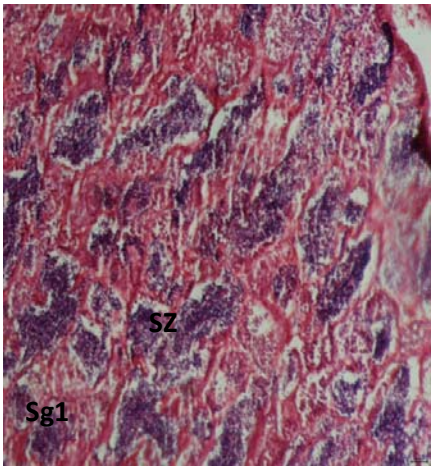

Fig. 10: Developing stage

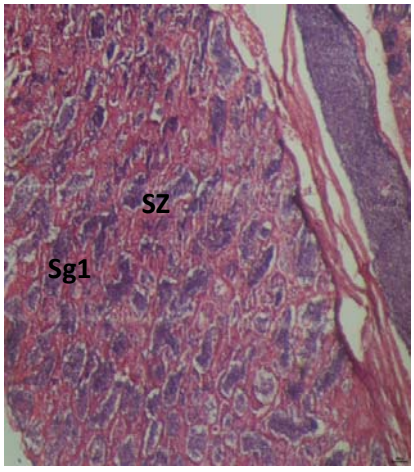

Fig. 11: Developing stage

Figure (12-17): Ovaries of *Oxyeleotris marmorata* at different stages of maturity

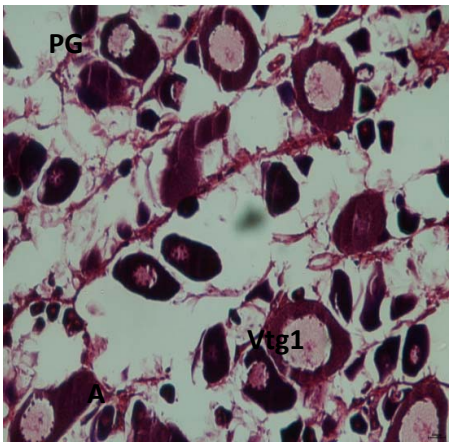

Fig. 12: Immature stage

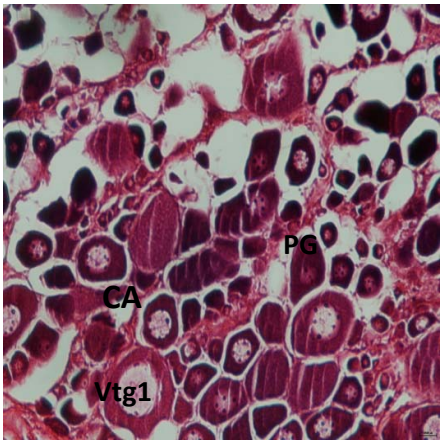

Fig. 13: Immature stage

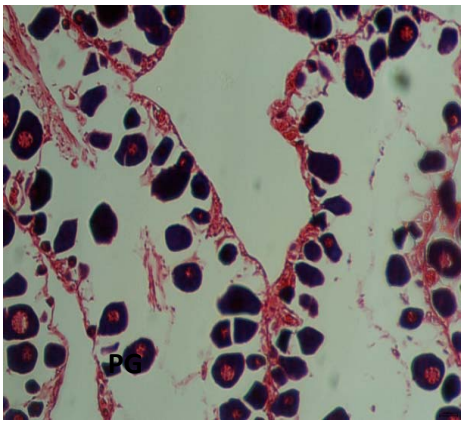

Fig. 14: Immature stage

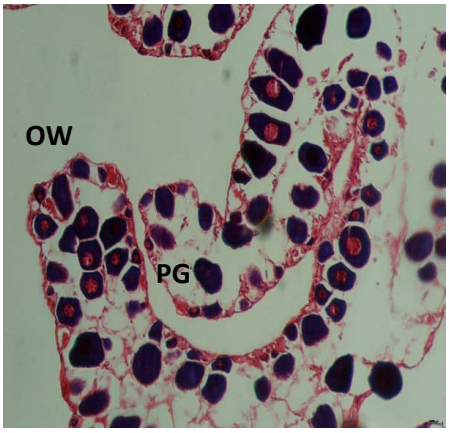

Fig. 15: Immature stage

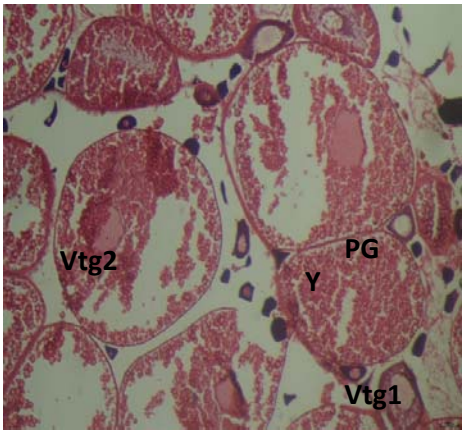

Fig. 16: Developing stage

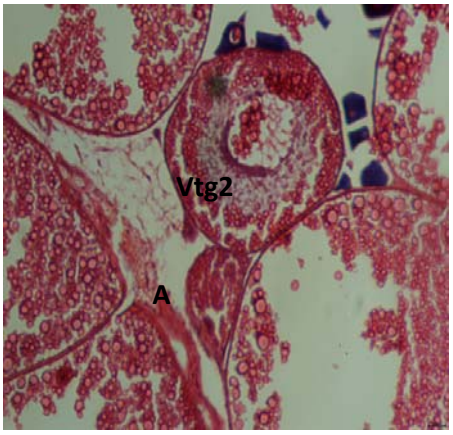

Fig. 17: developing stage

Discussion:

Sex determination in fish is a very complex and flexible process with respect to evolutionary patterns observed among genera and families, and within individuals is subject to modification by external factors. Sexual development in fish is characterized by many factors, including genetic and environmental, male and female heterogametes, single gene and polygenic systems, protandry, protogyny, social influence on sexual determination and many combinations and variations between and with these systems.

The determination of sexuality in fishes is vital to many fisheries biologists, researchers and aquaculture practitioners. Methods of sexual identification involving external morphological features can minimize stress and injury to the fish, faster and reliable to apply in the field than other available techniques. The result of this study indicated that urogenital length was slightly longer in female O. marmorata than in the male while caudal fin length and caudal peduncle were longer in male than female. These features can distinguish between the two sexes. However, the only characteristic which is useful practical to conduct on the field is urogenital length.

A myriad species of fish exhibit a sexually related dimorphic growth pattern in which the fish of one sex reaches a larger ultimate size than the other (Scott and Crossman, 1973; Becker 1983). Usually this feature is not visible in juvenile or young adult fish and slightly imprecise to be useful for field guide. Sex in many species of fish can be identified by the release of eggs or semen, or from abdominal distention caused by the larger size of ovaries compared with testes during spawning and prespawning season (Scott and Crossman, 1973; Becker 1983). Clearly such characteristics cannot be used outside of the spawning season or sexually immature fish.

In male and female red tilapias, sexual characteristics can be differentiated by the shape of the body and relative position of the genital papilla. The male has two orifices under its belly, one is the anus and the other is the urogenital papilla. The female has three, which are; the anus, the genital and urinary apertures. The anus is easily recognized as a round hole. The urogenital aperture of the male is a small point and the urinary orifice of the female is microscopic and is visible to the naked eye, while the urogenital orifice is an opening in a line perpendicular with the axis of the body (Beveridge and McAndrew, 2000).

In some species of fish that have few other sex-related external features, the differences in the external appearance of the urogenital region have been used to identify the sex of the fish. In a study conveyed by McComish (1968) in bluegills Lepomis macrochirus, the criterion of presence (in females) or absence (in males) of a large, swollen, scaleless area surrounding the urogenital opening has been used in sexual determination. In another study conducted by Parker (1971) the shape of the scale less area around the urogenital opening (elliptical or pear-shaped in females, round in males) has been applied to differentiate between the sexes in large-mouth bass Micropterus salmoides.

Sex determination techniques by using external urogenital morphology have been developed for some of the mesothermal (coolwater) teleosts, including members of the ecosid family such as northern pikes Esox lucius and muskellunge E. masquinongy. In northern pike, Casselman (1974) identified sex based on the presence or absence of an area of convoluted tissue in females and males respectively.

Furthermore, Ostrand *et al*., (1999) revealed sexual dimorphism in morphometric and meristic features other than the not so apparent of body coloration and nuptial tubercle that are normally used for sex identification in Hypognathus placitus (Plain minnows). It was found that the male Plain minnows predominantly have larger heads and peduncle region than the female. The inter-orbital length and the peduncle length are longer in the females than in the males. The females have such features for the spawning strategy which are used in mixing the eggs and the milt during fertilization, and the faning of the eggs during incubation to achieve adequate aeration and egg agitation (Lagler *et al*., 1962; Oladasu, 1997). However, the use of inter-orbital length and the peduncle length is not practical on the field because they are not easily observable and require measurements.

In species where sex determination from external morphology is difficult, gamete exteriorization with catheter or cannula is useful not only to determine the sex of the fish, but also to examine the readiness of the reproductive gamete. Numerous amount of study reported the environmental manipulation of the respective species to achieve both gonadal maturation and spawning success. Environmental, social and behavioural manipulations cues are ideal for induction of gonadal maturation and spawning in species where sex determination is difficult. Oladasu (1997) emulated the natural environment spawning conditions required by G. niloticus to achieve spontaneous spawning in the species. Those conditions included increase in water levels typical of the rainy season and the presence of suitable substrate as found in flood plains.

Jeffrey *et al*., 2011 used three parameters (the shape of the urogenital opening, the shape of the anterior edge of the urogenital opening when thumb pressure was applied to the abdomen and the presence or absence of swelling around the anus) in sexual identification of yellow perch Perca flavescens. In females the urogenital was crescent or half-moon shaped, its anterior edge remained linear when pressure was applied to the abdomen, and a ring-shaped area of swelling was present around the anus. While in males the urogenital pore was circular, its anterior end distended and the area around anus was not generally swollen.

In this study we have confirmed the female have longer urogenital papillae than in female. The female have longer urogenital based on the fact they have to deposit the eggs on the surface of substance or spawning during breeding activity. The findings of this study are consistent with Tan and Lam (1988). However, the shape of urogenital papilla in male and female were difficult to observe. In addition to that, we have found the caudal fin length and caudal peduncle length are longer in male than female. The males have such characters for the spawning strategy where male tends to guard the eggs after oviposition. Such features may be used for mixing the eggs and the milt during fertilization, and the faning of the eggs to obtain adequate aeration and agitation.

The elucidation of sex determination in marble goby will greatly help in broodstock management programme to achieve mega scale production.

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