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SEX DETERMINATION IN *Heterotis niloticus* (CUVIER 1829) BASED ON MORPHOMETRIC FEATURES

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ABSTRACT

This study examined the morphometric and meristic characters of African Bony Tongue (*Heterotis niloticus*) in order to identify possible phenotypic sex markers in this potential aquaculture species. The ratios of external measurements of the dorsal length, the pre-dorsal length, the head length, the peduncle length, the anal length, the pectoral length, the pelvic length and the caudal length to the standard length was determined. The inter-orbital length of head length and the snout length of head length were also analyzed. The analyses were done in thirty (30) specimens of *Heterotis niloticus*. Only two of the morphological ratios namely peduncle length of the standard length and the inter-orbital length of the head length indicated significantly higher values ($P<0.05$) in the females. The ratio of peduncle length to the standard length in the female was 5.13 ± 0.56 and 4.89 ± 0.43 was recorded in the male, while the ratio of inter-orbital length to head length in the female was 39.35 ± 2.16 and 37.54 ± 2.05 was recorded in the male. Sensory pits count on both the right and the left opercula plates were higher in the male than the female though, the difference was not significant enough for sensory pits count to form the basis for sexual dimorphism. The dimorphism so indicated in peduncle length and inter-orbital length is not readily observable by visual examination and may require simple measurements, which may not be easily carried out for routine sex determination on the field. However, it can be used to separate the broodstock population into different male and female holding facilities, while exteriorization of sexual products by catheterization or hand stripping can be used to confirm the sex of the selected broodstock and their readiness to spawn during the breeding season.

Keywords: Sex determination, morphometrics, *Heterotis niloticus.*

INTRODUCTION

Heterotis niloticus, the only specie of the family Osteoglossidae in tropical Africa, is of commercial importance in both culture and capture fisheries (Reed et al., 1967; Akegbejo-Samson, 1999; Idodo-Umeh, 2003). They are caught throughout the year with their contribution in landings being higher in the rainy season when water level rises; usually between June and August. Reed *et al.* (1967) reported that they are caught mainly in traps, seine nets and gill nets. Most often during the breeding season, they are caught together with their brood because of the parental care they exhibit. The stock depletion that can result from this qualifies the fish for the list of the endangered species.

The need to tackle the possible stock depletion, and the various attributes of the species including good growth rate, low nutrient requirement (herbivore), low lipid content of the flesh (Achionye-Nzeh and Omoniyi, 2002) that keeps the fish fresh even days after capture attest to its potentials as a viable aquaculture candidate. However, aquaculture production of any species is predicated on availability and adequacy of good quality seeds. The procurement of the seeds of *H. niloticus* still depends largely on collection from the wild. Up until now, efforts at controlled propagation of the species is limited to natural spawning induced by the simulation of the natural breeding environment in the ponds, that is, provision of grassy banks that simulate floodplain conditions in rainy season (Moreau, 1974).

The culture of *H. niloticus* will be enhanced with the development of techniques for controlled breeding. One major obstacle in this regard is the absence of distinct external feature to distinguish the male of the fish from the female. Broodstock selection is much more difficult, and controlled breeding more cumbersome without such distinguishing feature. This study reports the results of investigations of possible presence of apparent external sex features in *H. niloticus*.

MATERIALS AND METHODS

Collection of specimen

Adults *H. niloticus* were randomly procured from different sources including Otuocha fish market, by the river Niger in Anambra State, a fish farm in Ughelli, Delta State and fish mongers at the Yanga Fish market in Benin-City. The fish samples were stored in cold packs from points of procurement and transported to the Nigerian Institute for Oceanography and Marine Research Wet-Laboratory in Sapele, where all measurements and dissections were made.

Morphometric and meristic observations

The weight and length of each specimen were taken using a top-loading weighing balance and a meter rule, respectively. Morphometric and meristic parameters were made for each specimen as described by Teugels (1986). Morphometric measurements and meristic counts used are as shown in Fig 1.

The ratios of dorsal fin length, pre-dorsal fin length, head length, peduncle length, anal fin length, pectoral fin length, pelvic fin length and caudal fin length, to the standard length were measured and calculated. The same was done for the ratio of inter-orbital and snout lengths to the head length.

The only meristic feature counted is the sensory pits. The sensory pits of the cranium and the opercula plates were counted for all specimens.

Gonadal Examination

Subsequent to 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, 3m histological section of the gonad was made and stained with Bouin's Haematoxylin and Eosin, and observed under the microscope to determine the sex of the specimen.

Statistical analysis

The mean values of ten morphometric ratios and one meristic feature enumerated for the thirty specimens were statistically compared between identified male and female specimens using Students' t'- test.

RESULTS

The observed sex ratio was 1:1 (15 males and 15 females) for the thirty specimens of *H. niloticus* observed 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, both of which were females, weighed 0.55 and 4.05kg, and measured 41.0 and 80.5cm, respectively.

Gonadal examination was used to confirm the sex of the specimens. The gonads were observed to be single (unpaired) for both sexes and are located on the right side of the dorsal wall of the abdominal cavity as reported by Moreau (1982). Six (6) females were found to be gravid, therefore requiring no histological examination for sex identification. Nine other specimens were identified as females via histological examination, which was also used for the identification of the entire fifteen male specimens.

Physical examination of specimens revealed no striking morphological or meristic feature distinguishing the male from the female. All appendages looked exactly alike in shape, feel and location on the body for all the specimens.

Tables 2 and 3 show ten morphological ratios of the male and female specimens respectively. A comparison of these ratios, using Students't'- test of significance revealed significant difference in the observation made for only two of the ten morphological ratios (Table 4). The ratio of peduncle length to the standard length observed for male *H. niloticus* was 4.89 ± 0.43, which was significantly lower ($p<0.05$) than the 5.13 ± 0.56 observed for the female. The same applies to the ratio of inter-orbital length to head length. The 37.54 ± 2.05 observed for the male was significantly lower than the 39.35 ± 2.16 observed for the female. However, these were differences that cannot be readily observable grossly.

Furthermore, the number of sensory pits on the head of *H. niloticus* ranged from 40 to 47 as observed in this study. However, the count on the dorsal part of the head (cranium) was 15 and constant for all specimens. On the opercula plates (right and left) the count varied from one specimen to another, ranging from 8 to 12. The difference that was observed in the opercula sensory pit counts had no specific pattern. The mean sensory pit counts observed on the right and the left opercula of the male specimens were slightly higher than those observed in the females (Table 5). The difference was, however, not significant ($p>0.05$).

DISCUSSION

The result of this study showed that the peduncle length and the inter-orbital length were longer in female *H. niloticus* than in the male, and should distinguish between the two sexes. However, the observed differences were not visibly apparent and hence could not be easily used on the field. The implication of this fact is that controlled propagation will be difficult in the fish since gravid male and female fish must be identified for controlled propagation to be successful. Similar observations were made in the trunk fish, *Gymnarchus niloticus* (Oladosu, 1997) and the milk fish, *Chanos chanos* (Liao and Chen, 1983), where external sex characteristics were not apparent and sex determination was therefore difficult. All these underscore the importance of the use of the analysis of morphometric and meristic features as veritable tools for species identification and sex determination especially on the field. Various investigations conducted on the identification of phenotypic markers, (either morphometric or meristic) in fishes, emphasized the importance of such features in controlled propagation and field studies. For instance, Shehan *et al.* (1999) developed character index (CI) and morphological character index (MCI) from two meristic features and five morphological ratios respectively, to distinguish between two morphologically similar species of Sturgeon namely *Scaphirhynchus albus* (Pallid Sturgeon) and *Scaphirynchus platorynchus* (Shovel-nose sturgeon), and their hybrid. The two indices, which could readily be applied on the field, provided the much-needed solution to the problem of possible stock pollution.

Furthermore, Ostrand *et al.* (1999) investigated sexual dimorphism in morphometric and meristic characters other than the not so apparent body colour and nuptial tubercle that are commonly used for sex determination in *Hypognathus placitus* (Plain minnows). It was observed that the male Plain minnows predominantly have larger heads and peduncle region than the females. The observation is the reverse in *H. niloticus* as observed in this study. The inter-orbital length and the peduncle length are longer in the females than in the males. The female could possibly require bigger peduncle for the purpose of mixing the eggs and the milt during fertilization, and the fanning of the eggs during incubation to achieve adequate aeration and egg agitation (Lagler *et al*., 1962; Oladosu, 1997). The wider inter-orbital length could also achieve a wider optical view in guarding the nest pre- and post- spawning. However, just like the not so apparent body colour in the plain minnows, the use of inter-orbital length and the peduncle length may not be easily observable in differentiating between the male and the female *H. niloticus*, because it requires measurements.

In all species that sex determination from external features is difficult, gamete exteriorization with catheter or cannula may be required not only to determine the sex of the fish, but also to ascertain the viability of the reproductive gamete. This has been achieved for *G. niloticus* as reported by Oladosu (1997), and *Chanos chanos* (Liao and Chen, 1983). Both studies reported the environmental manipulation of the respective species to achieve both gonadal maturation and spawning. Environmental, social and behavioral manipulations are ideal for induction of gonadal maturation and spawning in species where sex determination is difficult. Oladosu (1997) simulated the natural environmental spawning conditions required by *G. niloticus* to achieve spontaneous spawning in the species. Those conditions included increase in water levels typical of the peak of the rainy season and the presence of ideal spawning substrate as found in flood plains. From the observation of Moreau (1974), similar conditions are required by *H. niloticus* whose spawning habit and habitat are similar to that of *G. niloticus*, though the spawning substrate differs.

The probability of equal representation of male and female fish in a randomly selected population observed in this study suggests the possibility of natural spawning, if the natural habitat conditions are simulated.

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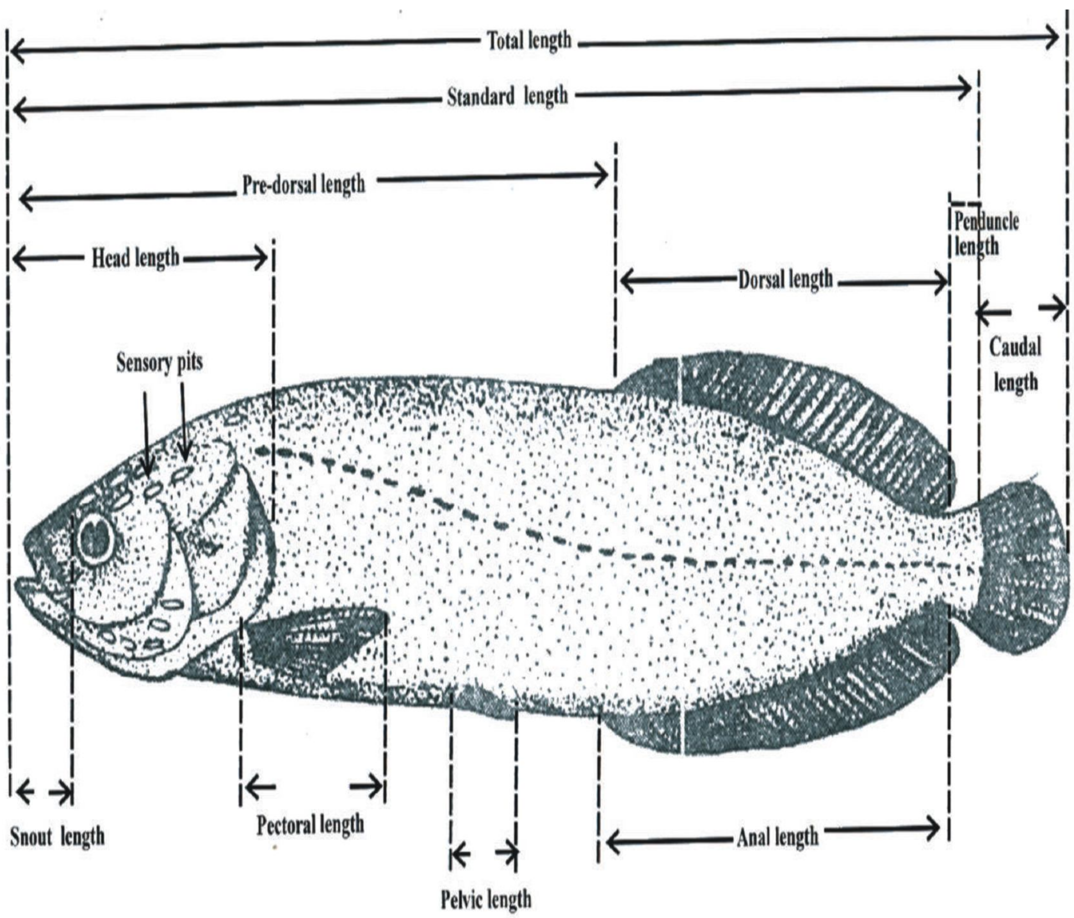

Fig. 1: *Heterotis niloticus* showing morphometric and meristic features

Table 1:Weight and Length of male and female samples of *Heterotis niloticus*

Parameter		Male	Female
Weight (kg)	***Range***	0.6 - 3.8	0.55 - 4.05
	Mean	1.66 ± 1.03	1.78 ± 1.34
Total length (cm)	***Range***	43.5 - 74.0	41.0 - 80.5
	Mean	56.5 ± 10.94	57.15 ± 14.61
Standard length (cm)	***Range***	39.4 - 69.0	38.5 - 74.0
	Mean	51.72 ± 10.63	52.16 ± 13.9

Table 2: Ratios of morphological characters of samples of male *Heterotis niloticus*

Morphological Ratio	n	Min.	Max.	Mean	Std
Dorsal fin length (%SL)	15	31.70	51.78	35.00	3.84
Pre-dorsal length (%SL)	15	55.38	66.25	62.37	2.95
Head length (%SL)	15	22.75	31.25	26.18	2.43
Peduncle length (%SL)	15	4.06	5.38	4.89	0.43
Anal fin length (%SL)	15	31.25	40.68	37.33	2.36
Inter-orbital length (%HL)	15	33.89	40.00	37.54	2.05
Pectoral fin length (%SL)	15	10.25	15.18	13.17	1.32
Pelvic fin length (%SL)	14	5.40	9.29	7.22	1.21
Caudal fin length (%SL)	14	6.15	12.11	9.69	1.64
Snout length (%HL)	15	18.64	28.36	23.27	2.70

n= Sample size Std= Standard deviation

Table 3: Ratios of morphological characters of samples of female *Heterotis niloticus*

Morphological Ratio	n	Min.	Max.	Mean	Std
Dorsal fin length (%SL)	15	30.69	38.57	34.32	2.10
Pre-dorsal length (%SL)	15	59.30	65.15	62.40	1.94
Head length (%SL)	15	23.29	28.92	26.06	2.07
Peduncle length (%SL)	15	4.11	6.06	5.13	0.56
Anal fin length (%SL)	15	29.73	40.54	37.54	2.57
Inter-orbital length (%HL)	15	34.48	42.73	39.35	2.16
Pectoral fin length (%SL)	15	10.60	16.28	13.20	1.52
Pelvic fin length (%SL)	14	4.55	9.64	7.07	1.39
Caudal fin length (%SL)	14	6.82	13.86	10.11	2.15
Snout length (%HL)	15	20.00	33.64	25.17	3.29

n= Sample size Std= Standard deviation

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

Morphological Ratio	Male*	Female*	Sig. diff. ($P < 0.05$)
Dorsal fin length (%SL)	35.09 ± 3.84	34.32 ± 2.10	ns
Pre-dorsal length (%SL)	62.37 ± 2.95	62.40 ± 1.94	ns
Head length (%SL)	26.18 ± 2.42	25.95 ± 1.74	ns
Peduncle length (%SL)	4.89 ± 0.43	5.13 ± 0.56	s
Anal fin length (%SL)	37.33 ± 2.36	37.54 ± 2.57	ns
Inter-orbital length (%HL)	37.54 ± 2.05	39.35 ± 2.16	s
Pectoral fin length (%SL)	13.12 ± 1.32	13.20 ± 1.52	ns
Pelvic fin length (%SL)	7.22 ± 1.21	7.07 ± 1.39	ns
Caudal fin length (%SL)	9.69 ± 1.64	10.11 ± 2.15	ns
Snout length (%HL)	23.27 ± 2.70	25.17 ± 3.29	ns

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

Table 5: Comparison of sensory pit count in male and female *Heterotis niloticus*

Meristic feature	Male*	Female*	Sig. diff. (P <0.05)
Sensory pits			
Right opercular	10.29 ± 2.87	9.8 ± 1.15	ns
Left opercular	10.00 ± 1.00	9.93 ± 1.33	ns

* mean ± standard deviation ns = not significant