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Aspects of the reproductive biology in mudskippers *Periophthalmus barbarus* (Gobiidae) (Linneaus 1766) in mangrove swamps of Iko Estuary, Southeast, Nigeria

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Abstract
Aspects of the reproductive biology of mudskipper, *Periophthalmus barbarus* (Gobiidae) in the mangrove swamp of Iko Estuary, Southeast, Nigeria, was studied between November, 2011 and October, 2013. Sexes were differentiated on the basis of the genital papillae which was broader in females than males. Sex ratio of 1 male; 1.3 female was observed which depicts a significant departure (x^2=175.598, 1df; P<0.001) from the theoretical 1 male: 1 female sex ratio indicating females were significantly more than males. The GSI values varied from 0.01 to 3.23% in males and between 0.02 to 4.01% in females with the mean value of 0.23±0.07% (in males) and 0.914±0.41% (in females) throughout the period of study. Mean HSI of males (1.372±0.358%) was significantly higher than that of the females (1.325±0.293). Condition index varied with sex, in males K varied between 0.17 to 1.56% with a mean value of 1.11±0.08% while that of the females varied from 0.25 to 1.62% with a mean value of 1.09±0.07. Mean K of the males was slightly higher than that of the females. Six (6) stages of maturity, immature, early development, late development, mature (Gravid), ripe and spent representing the pre-spawning, spawning and post-spawning phases were encountered in this study. The maturity stages obtained from the present study were in conformity with those reported in other teleosts though with modifications. Therefore, the sexual dimorphism, sex ratio, Gonadosomatic index, Hepatosomatic index, condition index and maturation of the species in the mangrove swamp of Iko Estuary was investigated to provide baseline data for future ecological and biological studies of this species and other species within the Nigeria mangrove swamps, estuaries and coastal waters.

Keywords: *Periophthalmus barbarus*, Sex ratio, Gonadosomatic index, Hepatosomatic index and Condition factor

1. Introduction
Periophthalmus barbarus belongs to the family Gobidea (FAO, 1990) [19]. It is the only reported species in the gulf of Guinea, which include West African Coast, estuaries and Lagoons (Irvine, 1847; FAO, 1990; Graham 1997) [25, 19, 22]. Members are bony and highly active fishes and their versatility is reflected in their euryhaline, euryphagous and amphibious nature (FAO, 1986; Lawson, 2010a) [18, 36]. It lives in the shallow and exposed inter-tidal mudflats of estuaries and mangrove swamps and it is amphibious (FAO, 1990) [19]. *P. babarus* is widely fascinating of all tropical fish (Jaafar *et al*., 2006) [27]. They can be seen jumping, climbing and skipping about with great agility in the estuarine swamps (King and Udo, 1997; Jaafar *et al*., 2009) [34, 28]. *P. barbarus* originated in West Africa and were found on the coastal areas between Angola and Senegal. The expected life span of *P. barbarus* is 5 – 8 years. The geographical range is between Senegal (St. Louis) and Angola (Luanda, River Quito), including Marcias, Sao Tome and Precipe Island (FAO, 1990) [19]. Other related species reported in some parts of the world include; *P. chrysospilos* which was reported in Singapore (IP *et al*, 1990) [24], and *P. koelreuteri* in East Africa, *Boleophthalmus boddaerti* and *B. woberi* are found inhabiting estuaries of Persia in Singapore. *P. barbarus* was reported as an indigenous or permanent element of the brackish waters of estuaries and Lagoons (Irvine, 1947; Udo 2002 a, b.; Lawson, 2010b, 2011) [25, 53, 54, 37, 38]. Aspects of the biology, ecology and early life history of mudskippers have attracted attentions of several authors (Murdy, 1989; Etim *et al*, 2002, Udo, 2002b; Jaafar and Larson, 2002; Khaironizam and Norma–Rashid, 2002; Swanson and Alice, 2004; Sarimin *et al*., 2009, Lawson, 2010) [44, 17, 54, 26, 29, 51, 49]. A new species, *Periophthal mustakita* was recently discovered in Australia by Jaafar *et al* (2006) [27].

2. Materials and Methods

2.1 Study Area

The mangrove swamp of Iko Estuary is located in Iko town, an oil producing community in Eastern Obolo Local Government Area, Akwa Ibom State, in the Niger Delta region of Nigeria (Fig. 1). The area lies within latitude $7^0 30^1$N and $7^0 45^1$N and Longitude $7^0 30^1$E and $7^0 40^1$E (Udotong *et al*, 2008) [55]. The river has a shallow depth ranging from 1 to 7m at flood and ebb tide. Iko river takes its rise from Qua Iboe river catchment and drains directly into the Atlantic Ocean at the Bight of Bony (Ekpe *et al*, 1995) [14]. The adjoining Creeks, channels and tributaries from the Iko River estuary is significant in the provision of suitable breeding sites for the diverse aquatic resources that abound that abound in the area, good fishing ground for artisan fishers as well as petroleum exploration and production activities (NDDC, 2004) [45]. The shoreline is fringed with mangrove and Nipa vegetation, tidal mudflat and pneumatophores of Avicennia exposed during low tide. The macrophytes are composed of the native red mangrove; *Rhizophora racemosa*, *R.harrizonii*, *R. mangle*, White Mangrove (*Avicennia africana*) and *Laguncularia racemosa* and the exotic Nipa (*Nipafrusticans*). (King, 1991; Ekwere *et al*, 1992; Ukpong 1995; Udo 1995; King and Udo, 1997; NDES, 2000) [31, 15, 57, 52, 34, 46]

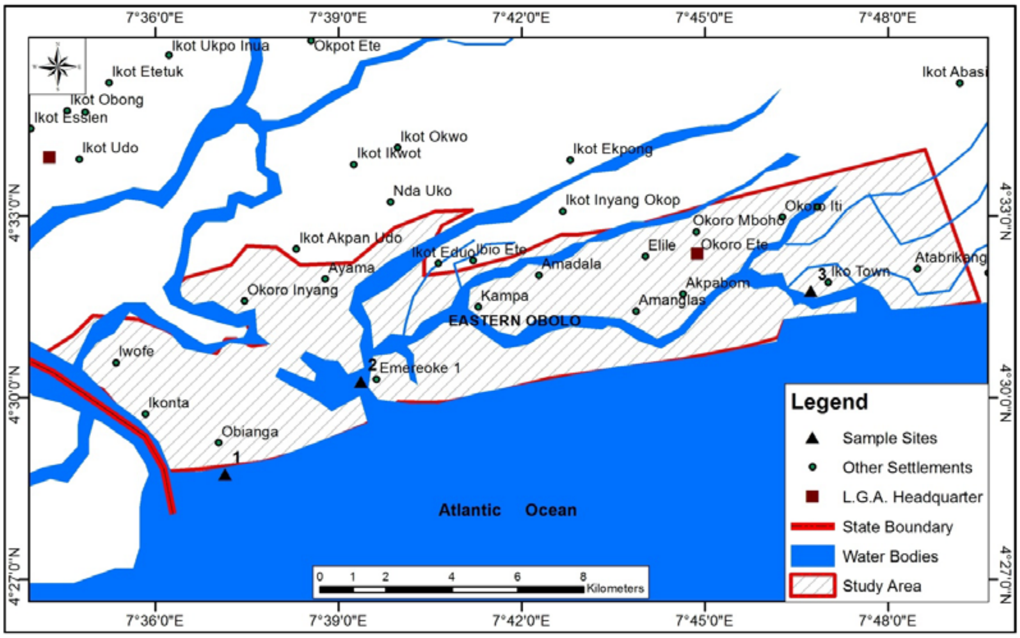

Fig. 1: Location of the sampling stations on the map of Eastern Obolo Local Government Area

2.2 Collection of Specimens

Monthly samples of the mudskipper, *Periophthalmus barbarus* were caught between November 2011 and October 2013 with non-return value traps from mudflats of the mangrove swamp of Iko Estuary. Services of local fishers were employed in setting up traps and diurnal collections of the fish. They were preserved immediately after capture in 10% formaldehyde solution prior to laboratory procedure.

2.3 Biometric data on total length (TL) to the nearest 0.0 1 cm and body weight measurement (TW) to the nearest 0.01g were recorded. Further laboratory analysis was carried out by opening bellies of the specimens to ascertain gonad weight and naked eye examination of the gonads. Methods of classifications of gonadal stages followed those of Blay and Eyeson (1982) [7] Marcus (1982) [40], Ugwumba (1984) [56], Udo (2002a) [53], Brutton (1979) [8], Kock and Kellerman (1991) [35].

The sexes were differentiated based on the observation of the genital papillae on the postero-ventral surface of the abdomen. They were broad and the free end More rounded in females. In males, the free end tapered and resembled the human tongue (Udo, 1995; 2002b) [52, 54]. The males and females were identified, counted and recorded. T-test statistics was used to evaluate sexual dimorphism in morphometric parameters (Baily 1959; Strum, 1978; Parker, 1979) [6, 50, 48]

Sex ratio was determined by counting number of male and female specimens. The ratios were tested with chi – squared analysis (X^2)

Each specimen was dissected and the ovaries and testes removed. Ovaries and testes (and Liver) from each specimen of each sex was measured to the nearest 0.01cm (TL) and weighed to the nearest 0.01 g. Somatic weight (SW) (g) of each specimen was calculated thus (Jaafar *et al*., 2006) [27]. SW = TW – G^0W, where TW = total weight of fish (g) and G^0W = Gonad weight (g). The gonadosomatic index was calculated according to strum (1978) as follows: GSI = G^0W/SW x 100.

Where G^0W = Gonad weight (g), SW = Somatic weight of fish (g). The annual gonadal cycle was determined from changes in gonad weight as indicated by the gonadosomatic index.

Hepatosomatic Index (HIS) was calculated thus:

HIS = $^{LW}/_{TW}$– LW ×100. Where LW= Liver weight;

TW= Total Weight of fish (g)

3. Results

3.1 Sexual Dimorphism

The sexes of *P. barbarus* could easily be differentiated on the basis of the genital papillae on the postero-vental surface of the abdomen. The papillae were broad and the free end more rounded in females than in males. In the males, the free end of the genital papillae tapered and resembled the human tongue. Sexually active females were slightly heavier than similarly-sized males.

3.2 Sex Ratio

In the current study, a total of 2,798 specimens of *P. barbarus* were collected from the mangrove swamp of Iko Estuary. This number comprised 1,234 (44%) males and 1,564 (54%)

females giving a male: female ratio of 1:1.3. A chi square (X^2) revealed a significant departure ($X^2 = 175.598$, 1df; $P < 0.001$) from 1 male: 1 female sex ratio indicating females were significantly more than males.

3.3 Gonadosomatic Index
In the present study, GSI of the matured specimens varied with sex (Table 1) between 0.001 and 3.23% (0.23±0.07%) in males and from 0.02 to 4.01% with a mean value of 0.914±0.41% in females.

Table 1: Summary of the GSI, HSI and K in males and females *P. barbarus* in Mangrove swamp of Iko Estuary.

		GSI				HSI				K	
	Min	**Max**	**Mean**		**Min**	**Max**	**Mean**		**Min**	**Max**	**Mean**
Males	0.01	3.23	0.23± 0.07	Male	0.01	5.74	1.372± 0.358	Male	0.17	1.56	1.11±0.08
Females	0.02	4.01	0.914±0.41	Females	0.02	5.74	1.325±0.293	Females	0.25	1.62	1.09±0.07

3.4 In this study, HSI of matured specimens (table 1) varied in males from 0.01 to 5.74% with a mean value of 1.372±0.358% and in females from 0.02 to 5.74% with a mean value of 1.325±0.293. Mean HSI of the males was slightly higher than that of the females.

3.5 Condition index of matured specimens varied with sex (Table 1). Male K varied between 0.17 to 1.56% with a mean value of 1.11±0.08% while that of the females varied from 0.25 to 1.62 with a mean value of 1.09±0.07. Mean K of the males was slightly higher than that of the females

3.6 In this study, six (6) maturity stages were observed in male and female *P. barbarus*. These were stage I - Immature, Stage II - early development, stage III – late development, stage IV – mature or Gravid, stage V – Ripe, stage VI – spent. Table 2 shows the macroscopic feature of the stages of testes and ovaries development of *P. barbarus*.

Table 2: Stages of Maturity of Male and Female *P. Barbarus* in Iko Estuary, Nigeria

	Description	
Maturity Status	**Male**	**Female**
Stage 1 (Immature)	Testes were tiny, translucent elongated threats close under vertebral column. There was no presence of accessory sexual organs.	Ovary was tiny elongate threat, close under vertebrate column. There were no accessory sexual organs.
Stage 2 (Early developing)	Test string, pale, slightly white in colour, flattened, 1-2mm broad, and lobed. They occupied 1% of the body cavity and accessory sexual organs were visible	Ovary very small, firm, pale-white
Stage 3 (Late developing or resting)	Testis small, write, serated edges, slightly fatter with pointed ends and occupied about ⅛th of the abdominal cavity.	Ovary small, graining, orange-yellow, a heavy network of vessels appeared laterally on the surface of the ovary wall. Yellowish Oocytes were slightly visible to naked eye through the ovary wall. The ovaries extended for about 60 – 80% of the abdominal cavity.
Stage 4 (Gravid or Mature)	Testis white, serrated edges, slightly fatter with pointed ends and occupied about 1/5th of the abdominal cavity.	Ovary yellow, large and selling the body cavity. Ovary wall clearly transparent and eggs visible to naked eyes through the ovary wall. The ovaries extended for about 60 - 80%vof the abdominal cavity.
Stage 5 (Ripe)	Testis fully swollen and multilobed and Testis white, serrated edges, slightly longer and broader. Testis fully and multilobed and occupied about ¼th of the body cavity.	Ovary yellow, larger with contour walls, turgid, distend body cavity. Eggs clearly distinct, when opened a number of eggs spill out.
Stages 6 (Spent)	Testis shrunk having discharged sexual products, the testis reduced in size and relatively small in size, white walls were hard in texture.	The ovaries were reduced in size, faccid but with tough and smooth with no granulation. The colour was pale yellow and the residual oocytes were present and visible through flabby wall. The gonad extend for about 50% of the abdominal cavity

4. Discussion
In the present study on the fish *Periophthalmus barbarus*, sexual dimorphism, sex ratio, Gonadosomatic index, Hepatosomatic index, condition index and stages of maturity were discussed in order to clarify some characteristics of its reproductive biology.

P. barbarus were sexed onnte basis of genital papilae (King, 1996) [32]. A similar sex differentiation was reported by Udo (2002b) [54] on the same species in the mangrove swamps of Imo Estuary. Lawson, (2010b) [37] on *Periophthalmus papilio* from mangrove swamps of Lagos Lagoon reported a similar sex differentiation. A similar sex differentiation was reported for the goby, *Coryphopterus nicholsi* in the temperate zone (Cole, 1982) [11]. Females were heavier than similarly-sized males apparently due to heavier ovaries vis-à-vis the testes (King, 1991; Chu *et al*., 1993; Udo, 2002b) [31, 9, 54].

The overall sex ratio was significantly female biased. Given that 1:1 sex ration indicates the absence of a sex-based dichotomy in longevity (Nikolsky, 1969;Udo, 2002b) [47, 54], the 1.0:1.3 males : females ratio of *P. barbarus* in the estuarine swamps of Iko River probably depicts that the females live longer than males (King, 1991, Udo, 2002b) [31, 54]. Additionally, this female preponderance demonstrated that sufficient females are always available to maintain a good population equilibrium (i.e., ability to maintain its numbers). From the overall sex ratio of *P. barbarus*, it can be reasonably conjectured that during spawning, males spend more time in the burrows or at the spawning grounds guarding eggs as in other gobiids (Gibson,1969, Cole 1982; Mkpanam,1989; Udo, 2002b; Lawson, 2010a) [21, 11, 42, 54, 36]. This behaviour probably makes them less vulnerable to capture on the mudflats of the mangrove swamps. The gonadosomatic index of the fish varied between 0.01 and 3.23% in males and from 0.02 to 4.01% in females. Females showed higher GSI values tan males (Table 1). This was an indication that less than 3.23% in males and 4.01% of female body mass were committed to gonad development of this species (Udo, 2002a; Lawson, 2010b; Chukwu and Deekae, 2011; Adebiyi, 2013) [53, 37, 10, 1].

The GSI had been used to describe the development of gonads in pike, *Esoxlucius* (Danilenko, 1983) [12]. GSI increases progressively with increased percentage of the ripe individuals towards the spawning seasons (Mohammed, 2010) [43]. The most common practice for determination of a species spawning season is the establishment of its GSI and the histological examination of the gonads (El-Greisy, 2000; Assem, 2000 and 2003; Honji *et al*, 2006) [16, 3, 5, 23].

The hepatosomatic index (HSI) which is an intrinsic factor for determining stored up energy is affected not only by food availability but also in a population by its composition as matured populations have higher values of HSI (Katarzyna and Sapola, 2005, Lawson, 2010a) [30, 26]. Closely linked with reproduction and phenology of storage is annual variation in liver mass which could reflect the process of storage and transfer of energy from periods of intensive feeding to periods of restricted feeding but high energy demand (Miller, 1979, Udo, 2002b; Lawson, 2010a) [41, 54, 36]. In this study, the adequate and/or constant food resources in the mangrove probably evoked the similarity in HSI of the sexes of *P. barbarus* (Table 1); a design that is connected to hepatic energy storage for future uses.

Interpopulational variability in mean condition index was higher in males (1.11 ±0.08%) than females (1.09±0.07%). The instability in the body condition of males of *P. barbarus* in the mangrove, highlights perhaps energy requirement by the males in mate-pairing and guarding of eggs in the burrows, which most frequently in gobiids is male specific (Gibson, 1969,; Udo, 2002b) [21, 54]. Lizama and Ambrosio (2002) [39] had emphasized that the most important factor that affects the condition factor of fish is its reproductive activities. Similar work by King and Udo (1996) [33], gave the condition factor of *P. barbarus* in Imo River for males and overall as 1.19 and 1.01 respectively. Although the paper reported the highest K values for males in March and females in January, this work recorded highest K values for males in November (2011) and August for females. Chukwuand Deckae (2011) [10] on the same species in New Calabar river reported the highest K values for both sexes in September.

In this study six stages of gonad development were observed (Table 2). These stages were classified as:

1) Pre-spawning class which was represented by immature, early development and late development stages;
2) Spawning represented by gravid (mature) and ripe stages;
3) Post-spawning class which was represented by spent period of the fish in the mangrove swamp.

Observations of these stages of maturation in the fish though with modifications were in conformity with that of most teleosts (Assem, 2002 and 2003) [4, 5]. The presence of spermatocytes and oocytes at different stages of development was an indication that this species belongs to the fish with prolonged and fractional spawning season. Therefore, the fish may spawn more than once during the spawning season.

These observations of the different stages were in agreement with the reports of El-Greisy (2000) [16] on *Diplodus sargus*, Udo (2002b) [54] on *P. barbarus*, Honji *et al* (2006) [23] on *Merluccius hubbsi*, Garcia Diaz *et al* (2006) [20] on blacktail comber, *Serranus atricauda*, Dinh *et al* (2007) [13] on the Goby, *Pseudapocryptes elongatus*, Lawson (2010b) [37] on *Periophthalmus pailio*, Al-Nahdi *et al* (2010) [2] on *Pomadasys commersonnii*, Mohammed (2010) [43] on *Merluccius merluccius* and Adebiyi (2013) [1] on Sompat Grunt *Pomadasis jubelini*.

5. Conclusion

The current study on mudskippers, *P. barbarus* in mangrove swamp of Iko Estuary, Southeast, Nigeria showed that sexes could easily be differentiated on the basis of the genital papillae on the postero-ventral surface of the abdomen. The papilla was broad and the free end more rounded in females than in the males. A higher number of females than males (sex ratio of 1 male: 1.3 females). This was significantly different and a departure from the theoretical 1 male: 1 female ratio indicating that females were significantly more than males. The females recorded a higher GSI than males. In males, GSI ranged from 0.01 to 3.23% (0.23±0.07%) and from 0.02 to 4.01% with a mean value of 0.914±0.41% in females. Mean hepatosomatic index of the males (1.372±0.358%) was slightly higher than that of the females (1.325±0.293).

Mean condition index of males (1.11±0.08%) was slightly higher than that of the females (1.09±0.07%). There were six (6) stages of maturity representing the pre-spawning, spawning and post spawning periods of the fish in the mangrove swamp of Iko Estuary. This research will contribute valuable data necessary as baseline data for carrying out further ecological and biological studies for proper management, conservation of fisheries resources and increase the knowledge of reproductive biology of *P. barbarus*.

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