

Aspects of Reproductive Biology in Mudskipper, *Periophthalmus papilio* from Mangrove Swamps of Lagos Lagoon, Lagos, Nigeria

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Abstract: The present study was conducted on aspects of reproductive biology of mudskipper, *Periophthalmus papilio* (Bloch and Schneider 1801) in the mangrove swamps of Lagos lagoon in Nigeria. *P. papilio* is an economically valued fish species both as food for the populace and baits for industrial or offshore fisheries. About 2167 individuals were caught from the lagoon with non return valve traps between May 2006 and May 2008. Data on sex, Total Length (TL), gonad (gW) and body Weight (bW) measurements were carried out on individual specimen. These data were used in the determination of sex ratio, Gonadosomatic Index (GSI) and maturation in this species. The sex ratio of 1 male to 1.42 females was observed but not statistically different but a departure from the theoretical 1 male:1 female sex ratio. The females were significantly more than the males. The eggs were as small as 0.20 mm and as big as 0.50 mm (mean = 0.36 ± 0.01 mm for average fish of 116 TL). The GSI values varied from 0.01-0.48% in males and between 0.11 and 8.40% in females. Seven stages of maturity: immature, immature and developing, ripening, ripe, ripe running, spent and resting and recovering were encountered. Male fish matured at 70 mm TL and females at 60 mm TL. However, both sexes were at the ripe stage at 90 mm TL. In conclusion, females were significantly more than the males. Between 0.01 (in males) and 8.40% in (females) of the body mass was useful in gonad development. The maturity of females was earlier than the males. The maturity stages obtained from the present study were in conformity with those reported in other teleosts. Therefore, the sex ratios, egg diameters, gonadosomatic index and maturation of the species in Lagos lagoon were investigated to provide baseline data for future ecological and biological studies of this species and other species within the estuaries and coastal waters.

Key words: Atretic, fecundity, gonadosomatic index, oocyte, spermatocyte, Nigeria

INTRODUCTION

The gobies including the mudskipper, *Periophthalmus papilio* is made up of a large number of species. They are bony and highly active fishes and their versatility is reflected in their euryhaline and amphibious nature. *P. papilio* belongs to the family Periophthalmidae but formally of family Gobiidae. It is the only species found in the Gulf of Guinea including West African coast, estuaries and lagoons (Irvine, 1947; FAO, 1990) it lives in the shallow and exposed inter tidal mudflats of estuaries mangrove swamps and it is amphibious (FAO, 1990). Other related species found in other parts of the world include *P. chrysopilos* in Singapore (Ip *et al.*, 1990) and *P. koelreuteri* in East Africa. *Boleophthalmus boddarti* and *B. woberi* are found inhabiting estuary of Pasir Ris in Singapore. *Periophthalmus* was grouped into indigenous or permanent element of the brackish waters of estuaries and lagoons (Lawson, 2004a). Aspects of the biology, ecology and early life history of mudskippers have attracted attentions of several researchers (Murdy, 1989;

Etim *et al.*, 2002; Jaafar and Larson, 2008; Khaironizam and Norma-Rashid, 2002; Udo, 2002; Lawson 2004a, b; Swanson and Alice, 2004; Sarimin *et al.*, 2009). A new species, *Periophthalmus takita* was recently discovered in Australia (Jaafar *et al.*, 2006).

Study area-Lagos lagoon: Lagos lagoon (Fig. 1), Nigeria is located on longitude $3^{\circ}20'$ and $3^{\circ}50'W$ and latitude between $6^{\circ}24'$ and $6^{\circ}36'N$. It is the largest of lagoon systems in West African coast covering 208 km². It is an open tidal estuary. The lagoon is fed in the north by Ogun river which is the major source of water to the lagoon; the southern margin is bounded by 5 cowries creeks. The Badagry creek is the main waterway leading to Badagry and Nigeria/Benin Republic border. Connected with 5 cowries creek is kuramo water which is a channel which runs through the mangrove swamps. The lagoon opens into the Atlantic Ocean via the Lagos harbour. The adjacent Lekki and Epe lagoons bounded it in the east. Previously, several studies were conducted on benthic, fishes and fisheries of this water body (Olaniyan, 1957;

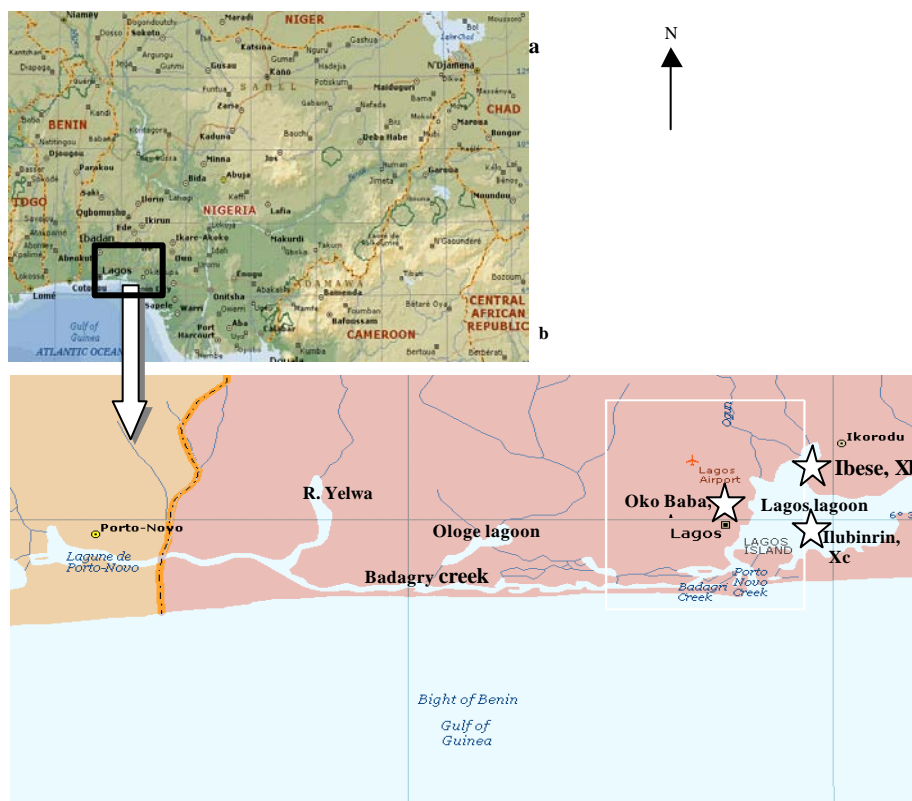


Fig. 1: (a) The administrative map of Nigeria (b) Inset Lagos lagoon showing the sampling stations (Xa, Xb and Xc)

Oyekan, 1972; Fagade and Olaniyan, 1972, 1973; Yoloje, 1976; Kusemiju *et al.*, 1983; Ajao, 1990; Brown, 1991; Lawson, 1991, 1998).

MATERIALS AND METHODS

Collection of specimens: About 2,167 specimens of mudskipper, *Periophthalmus papilio* were caught from mudflats of the mangrove swamps of Lagos lagoon between May, 2006 and May, 2008 with non-return valve traps. Services of local fishermen were employed in setting up traps and diurnal collections of the fish. Specimens were preserved in 4% formaldehyde prior laboratory procedures.

Laboratory procedures and data collection: Biometric data on Total Length (TL) to the nearest 1 mm and body weight measurements (bW) to the nearest 0.01 g were recorded. Further laboratory analysis was carried out by opening bellies of the specimens to ascertain sex and gonad weight (gW).

The maturity stages were determined by naked eye and microscopic examinations of the gonads. Methods of classifications of gonadal stages followed those of Jone (1974), Blay and Eyeson (1982), Marcus (1982) and Ugwumba (1984). Sex ratio was determined by counting

numbers of male and female specimens. The ratios were tested with Chi-squared analyses (χ^2). The annual gonadal cycle was determined from the changes in gonad weight as indicated by the Gonadosomatic Index (GSI). This was estimated from the relationship:

$$GSI = gW \cdot 100 \cdot bW^{-1}$$

The relationship between gW and bW of the fish was obtained from equation: $gW = a + b \cdot bW$. Where y = gonadosomatic index, x, w = body weight (g). Parameters a and b were estimated from the least squares regression method. Egg size or oocyte diameter (od) was measured with a graduated micrometer that was mounted in the eyepiece of a binocular microscope. The regression coefficient, r was calculated to the nearest 0.01 mm. The relationship that existed between and fish TL was determined.

RESULTS AND DISCUSSION

Sex ratio: A total of 2167 specimens of *P. papilio* were collected from the mangrove swamps of Lagos lagoon. This number comprised 281 immature, 779 male and 1107 female individuals giving a sex ratio of 1 male: 1.42 females. A Chi-square (χ^2) revealed a significant departure

from 1 male:1 female sex ration ($\chi^2 = 57.04 > \chi^2_{1,0.05} = 3.84$), indicating females were significantly more than males.

Gonadosomatic index: The values of GSI in this study are shown in Table 1. In males it ranged between 0.01 and 0.48% (0.104±0.004%) and in females from 0.11-8.40% (2.69±0.003%). The gonad weight-total length relationships in both sexes of *P. papilio* in Lagos lagoon (Fig. 2 and 3). For males:

$$gW = -0.0434 + 0.0005TL \quad (n = 126, r = 0.4385)$$

For females:

$$gW = -0.7502 + 0.0106TL \quad (n = 199, r = 0.3835)$$

Egg diameter: In this study, 4 different groups of egg sizes were observed in the lagoon viz: 0.2, 0.25, 0.33 and 0.50 mm in diameters representing 15.56, 12.06, 45.73 and 26.63 %, respectively (Fig. 4). The mean egg diameter was 0.36±0.002 mm. The relationship between oocyte diameter (od) and fish Total Length (TL) is shown in Fig. 5 as: $od = 0.1685 + 0.0014TL$ (n = 199, r = 0.2154).

Stages of maturity: Seven maturity stages were encountered in the study. Both microscopic and macroscopic descriptions of the maturity stages for the males and females in this study are shown in Table 2.

Size at maturity: Length frequency histograms of fish at different stages of maturity are shown in Fig. 5. Stage II specimens measured between 70 and 150 mm TL for males and females, respectively. The modal length frequency of 100 mm TL was by 26.52 of males and 30.44% of females (Fig. 6).

Stage III specimens were 80-160 for males and 90-190 mm TL for females (the modal length was 120 mm). However, 25.0 and 23.01% of males and females, respectively made up the modal length. Stage IV fish measured between 90 and 180 mm TL (for males) and 90-160 mm TL (for females), the modal lengths were 140 and 120 mm TL, respectively. The cohorts were 17.53 and 23.53% males and females, respectively. Stage V specimens occurred between 90 and 160 mmTL (males) and from 100-180 mmTL (females). At 120 mm TL, most females were already at ripe (IV) and ripe running (VI) stages earlier than males (140 mmTL). The fish in stage VI ranged from 100-160 mm TL. The percentages length frequencies for the cohorts were 26.53 and 24.62%, respectively for males at 150 mm TL and females at 130 mm TL. At Stage VII, 60% of the specimens were 130 mm TL. The females were between 110 and 160 mm TL, 28.57% of them measured 120 mm TL. At 90 mm TL both sexes attained their ripe stage.

Table 1: Summary of the GSI in male and female *P. papilio* in Lagos lagoon

Sex	Range (%)		Mean ±S.E.(%)
	Min	Max	
Males	0.01	0.48	0.104±0.004
Females	0.11	8.40	2.69±0.03

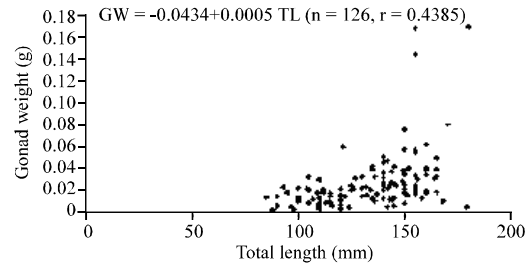


Fig. 2: Gonad weight-total length relationship in male *P. papilio* from mangrove swamps of Lagos lagoon, Nigeria

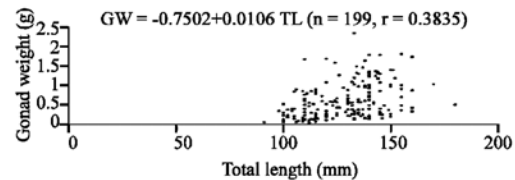


Fig. 3: Gonad weight-total length relationship in female *P. Papilio* from mangrove swamps of Lagos lagoon, Nigeria

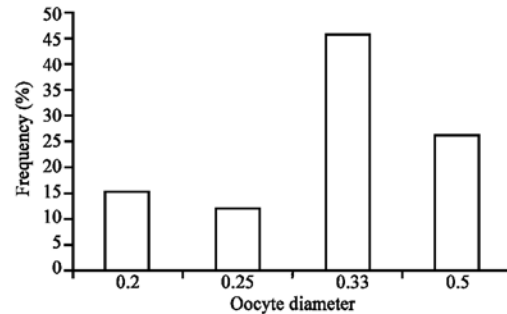


Fig. 4: Percentage frequency distribution of Oocyte diameter in *P. papilio* in Lagos lagoon, Nigeria

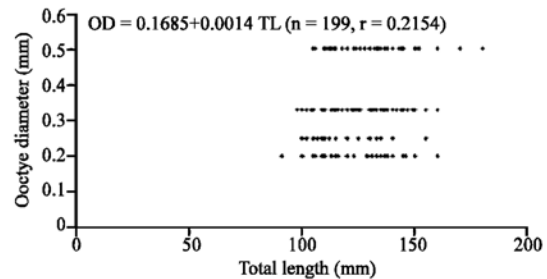


Fig. 5: Relationship between Oocyte diameter and fish total length of *P. papilio* in Lagos lagoon, Nigeria

Table 2: Summary of different stages of maturity of *P. papilio* from mangrove of Lagos lagoon, Lagos, Nigeria

Maturity stages	Males	Females
Stage I presence (Immature)	The abdominal cavity. Macroscopic examination did not reveal presence of accessory sexual organs which were associated with males. The microscopic examination showed no sex differentiation. The cells associated with the gonads were rudimentarily developed and could not be differentiated. Hence, the specimens were classified as immature	The abdominal cavity. Macroscopic examination did not reveal of accessory sexual organs which were associated with females. The microscopic examination showed no sex differentiation. The cells associated with the gonads were rudimentarily developed and could not be differentiated. Hence, the specimens were classified as immature
Stage II (Immature and Developing)	Macroscopically, the testes were flattened and 1-2mm broad whitish and lobed. They occupied 1% of the body cavity and the accessory sexual organs were visible. Testicular wall was thick with primary spermatocyte predominating peritoneum. The mesothelium of the peritoneum was very thick. The stoma and interlobular septa were very conspicuous	External or macroscopic appearance of the ovaries showed that they were small and rounded with a rough surface and soft texture. They were creamy in colour and measured between 1/8th (12.5%) and 1/4th (25.0%) of the length of the abdominal cavity. They were translucent with blood vessels forming internally; none of the oocytes were visible through the ovary wall. Histological appearance was characterized by the presence of many oocytes very conspicuous (0.025-0.05 mm). The larger oocytes had cytoplasm vacuoles. The ovary wall was thick at 50 µm and folded. The oocytes were irregularly shaped but few were rounded
Stage III (Ripening)	In early stage (phase 1) of ripening, the testes became fatter, off white and occupied 1/8th of the abdominal cavity. Blood vessels were visible through testis wall. Gonad length: width ratio was 2.8. Primary spermatocytes and spermatids were pronounced. In the late stage (Phase 2) of the ripening, the testes became firm and whiter and occupied 1/5th of the abdominal cavity. Lobulation of the right and left testes started. The length: width ratio was 2.4. The accessory sexual organs were of equal length with the testes. The secondary spermatozoa were present. The septa were not fully organized	The ovaries were swollen and lobed when observed macroscopically. A heavy network of vessels appeared externally on the surface of the ovary wall. Yellowish oocytes were visible to naked eye through the ovary wall. The gonad extended for about 60-70% of the abdominal cavity. The histological observation of the ovaries at this stage showed that many oocytes of between 0.1 and 0.2 mm were shown. Larger oocytes with cytoplasmic vacuoles were very few and had small yolk droplets. The primary and secondary vitellogenic oocytes dominated while tertiary vitellogenic oocytes were represented in the gonad. The cytoplasm of larger oocytes was filled with densely staining yolk granules. The ovarian wall was 70 µm thick
Stage IV (Ripe)	The testes were fully swollen and multilobed at this stage but did not occupy more than 1/4th of the body cavity. The colour was creamy white. The accessory sexual organs grew past the testes. The secondary and tertiary spermatocytes were dominant while few primary spermatocytes were represented. The testicular wall was 30 µm. The testicular septa were well organized and distinct	External appearance showed that the ovaries at this stage were almost filling the body cavity occupying 80-90% of abdominal cavity. The shedding of eggs has not commenced and otherwise soft. The eggs were rounded with a rough granular surface given a hollow sac like appearance. Blood vessels coalesced to form larger ones on the external surface of the ovary wall. Yellowish colour was possible due to the large yellow oocytes that were visible through ovary wall. The histological observation of the gonads showed that the secondary and tertiary vitellogenic oocytes dominated the gonad with very few primary oocytes. The theca externa were prominent. The hyaline oocytes were present but usually collapsed by histological processing. Ovary wall was 90µm thick; many oocytes were between 0.2-0.5 mm indiameter but usually 0.35 mm in size. Many oocytes were at stages II and III. There were blood vessels internally but some of the yolk oocytes were atretic
Stage V (Ripe running)	The testes were broadest, mostly firm but some were flaccid and highly lobulated. They were completely white but the posterior tip sometimes grew with speckled appearance. The accessory sexual organs were fully developed and became longer than the testes. No blood vessels and thick milt exuded on slight pressure before preservation. Testis length: width ratio was 2.2 and it extended for 50% of the abdominal cavity. The lumen contained spermatozoa. The empty spaces in the lumen also contained spermatocytes and spermatozoa. Most of the spermatozoa migrated towards the periphery of the lobule and the more advanced stages such as primary and secondary spermatocytes and the spermatids were found towards the interior. The testicular wall reached 30 µm	The oocytes or eggs flowed from the vent on slight pressure and the ovary occupied 99% of the abdominal cavity and rendered alimentary canal and gut almost inconspicuous. Oocytes looked exactly like those in the ripe stage and were laid singly with space (septata) in between as shown in most of the oocytes were in their tertiary vitellogenic stage
Stage VI (Spent)	The testes reduced in size and sometimes very small, flaccid and walls were hard in texture. They were dark brown colour and no blood vessel visible. Milts were absent, testis length: width ratio was 3.2 and gonad extended for 30% of the abdominal cavity. Wall reached 40 µm. The accessory sexual organs well developed and longer than what in stage V. The septa disappeared and mesothelium was thickest	The ovaries were reduced in size, flaccid but ovary wall was tough and smooth with no granulation. The colour was dark red. The residual oocytes were present and visible through flabby wall. The ovary length: width ratio was 4.5 and gonad extended for 50% of the abdominal cavity. The diameters of the oocytes were very few and had a thin densely staining cytoplasm. A few atretic residual oocytes were present and invasion of oocytes by follicular cells was noted. There was a

Table 2: Continue

Maturity stages	Males	Females
Stage VII (Resting and recovering)	Dark patches visible through the testis wall. The testes were about a third of length of body cavity or less and firmer than what obtained at spent stage and 3 mm broad. A big cavity was seen at the center of the gonad spermatozoa were present at the lumen. The mesothelium of theand residual peritoneum thickened	dense network of blood vessels which indicated a high level of oocyte atresia. The septum was disorganized and there was no empty follicular coat. The ovary wall was 300 µm thick. The lumen of the ovary contained much debris that was residual cells Externally, ovaries were firmer than spent stage but mainly red in colour. It occupied 60% of the body cavity and none of the residual oocytes oocytes were visible through the ovary wall sometime present The residual atretic oocytes were present when observed microscopically but the septum was not very organized. Reorganization of ovigerous lamellae started. A few reabsorbing oocytes were sometimes present

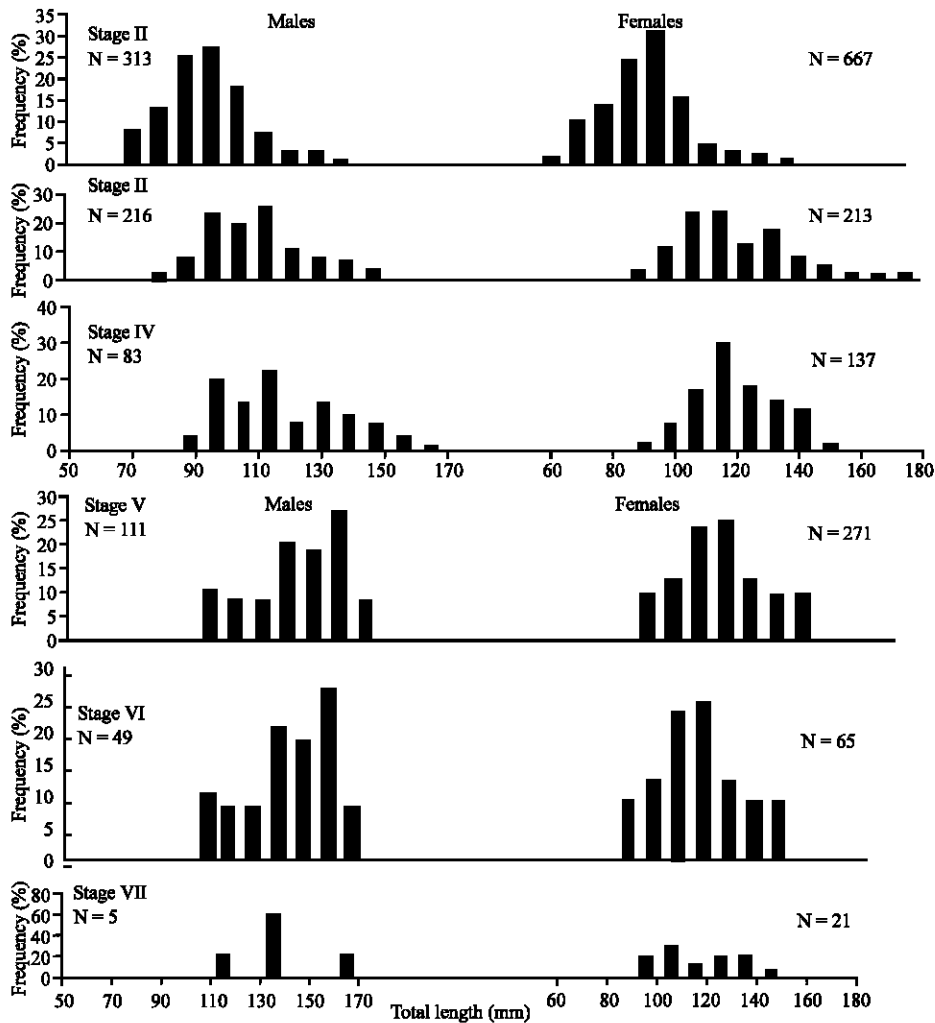


Fig. 6: Length frequency distribution of *P. papilio* at different maturity stages (II-VII) in the mangrove swamps of Lagos lagoon, Nigeria

In the present study, on the fish *Periophthalmus papilio*, sex ratio, gonadosomatic index, egg diameter and stages of maturity were discussed in order to clarify some characteristics of its reproductive biology. The sex ratio of this species in the mangrove swamps of Lagos lagoon indicated that females were significantly more than the

males giving a sex ratio of 1 male: 1.42 females. This is a departure from theoretical 1 male to 1 female sex ratio. Higher percentages of male to female ratio in favour of the former during the spawning period was reported in non related species like *Elop lacerta* (Ugwumba, 1984; Lawson and Aguda, 2010) and *Chrysichthys walkeri*

(Kusemiju, 1976) and while ratios in the favour of latter were reported in *Ethmalosa fimbriata* (Fagade and Olaniyan, 1972; Blay and Eyeson, 1982) and *Mugil cephalus* (Lawson, 1991) in some West African lagoons. The diameters of eggs in *P. papilio* ranged between 0.2 and 0.50 mm (mean = 0.36 ± 0.002 mm). The most prominent was 0.30 mm and the least was 0.25 mm egg diameters. This was in supports of the reports by Jone (1974) on *Scophthalmus maximum* and Marcus (1982) on *Ilisha africana*. The egg size in teleosts varies with the size of female (Thorpe *et al.*, 1984) with food supply (Bagenal, 1969) and from species to species (Bagenal, 1969; Hay and Brett, 1988).

The gonadosomatic index of the fish varied between 0.01 and 0.48 in males and from 0.11-8.40% in females. Females showed higher GSI values. The GSI had been used to describe the development of gonads in Pike, *Esox lucius* (Danilenko, 1983). This was indication that <0.48 in males and 8.40% of female body weights were committed to gonad development by this species. GSI increases progressively with increased percentage of the ripe individuals towards the spawning seasons (Mohamed, 2010). 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, 2003; Honji *et al.*, 2006).

In this study, seven stages of gonad development were observed. These stages represented the pre-spawning (immature, immature and developing, ripening); spawning (ripe and ripe running) and post-spawning (spent and recovering) periods of the fish in Lagos lagoon. These stages were in conformity with that of most teleosts (Assem, 2002, 2003) although with slight modification in this study. The presence of spermatocytes and oocytes at different stages of development indicate 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. This was in agreement with reports of Salem *et al.* (1994) on *Mugil seheli*, El-Greisy (2000) on *Diplodus sarus*, Honji *et al.* (2006) on *Merluccius hubbsi*, Garcia-Diaz *et al.* (2006) on blacktail comber, *Serranus atricauda* and Mohamed (2010) on *Merluccius merluccius*. Maturation process in *P. papilio* was similar to that of its relative, *Boleophthalmus pectinirostris* in Midori river, Japan.

Thus, maturation proceeds in this species as follows: in females, a portion of the oocytes in immature ovaries develop to the secondary yolk stage and form an oocyte group. Then parts of this oocyte develop further in turn to be spawned, leaving the remainder of the oocyte group

at the secondary yolk stage. At repetition of this process, spawning comes to an end with the declination of oocyte development in the ovary. In males, the spermatids develop to primary spermatocytes which further divide to secondary spermatocytes which to be spawned as spermatozoa. Like in females, repetition process makes spawning to come to an end with declination of milt development in the testis.

CONCLUSION

The present study on mudskippers, *P. papilio* in Lagos lagoon, Nigeria showed a record of higher number of females than males (sex ratio of 1 male:1.42 females). This was significantly different and a departure from the theoretical 1 male:1 female ratio. The smallest eggs measured 0.20 in diameter while the biggest were 0.50 mm (mean = 0.36 ± 0.002 mm).

Seven stages of maturity representing the pre-spawning and post spawning stages of the fish were observed. At 90 mm TL both sexes attained ripe stage, spent between 100-160 mm TL. Therefore, data from this research will be useful as baseline data for carrying out further ecological and biological studies for proper management and conservation of fisheries resources of Lagos lagoon.

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