# Testicular maturation and reproductive cycle in mudskipper, *Periophthalmus papilio* (Bloch and Schneider 1801) from Lagos lagoon, Nigeria

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**Abstract:** A study was carried out on mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria to determine its testicular maturation and reproductive cycle. *P. papilio* is a commercial valued fish in Nigeria as food for man and baits in capture fisheries, making its population in Lagos lagoon to be threatened. Therefore, conservation of its fishery from overfishing and exploitation is urgently required. A total of 796 male individuals were captured with non return valve traps between July 2004 and July 2006 from mangrove swamps of Lagos lagoon. They measured between 37 and 180 (104.83±25.57) mm TL and weighed 1.5 – 60.9 (18.60±10.65) g BW respectively. The testes were morphologically examined by naked eye and processed by standard histological techniques. ICES, BITS and IBTS scales and Bucholtz manuals were employed in the classifications of its maturity and gonadal stages. Seven reproductive stages were encountered in the study viz. immature, immature and developing, ripening, ripe, ripe running, spent and recovering-spent. The reproductive cycle included pre-spawning, spawning and post-spawning phases. The testicular maturation and reproductive cycle in mudskipper, *P. papilio* though with modifications were similar to what obtained in other teleosts. The GSI values ranged between 0.01 and 0.48 (0.132±0.165) i.e. less than 0.48% of the body weight was converted to development of testes. GSI values were at different peaks in July (0.23±0.016) and September (0.30±0.13%) 2004; May (0.198±0.004) and October (0.097±0.009%) 2005; and January (0.865±0.12), April (0.122±0.009) and July (0.145±0.016%) 2006 indicating the species as a multiple and synchronous spawner in Lagos lagoon. The study therefore provides the basic life history information on *P. papilio* through an objective approach in the assignment of maturity stage, using histological technique and macroscopic evaluations of the testes.
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**Key words:** Gonadosomatic index, spawning, spermatocyte, spermatid, spermatozoon, mudflat

## 1. Introduction

The present study which investigates the testicular maturation and reproductive cycle in mudskipper, *Periophthalmus papilio* from Lagos lagoon in Nigeria, is a third contribution in the study of reproductive biology of this species. The first and second were documented in Lawson (2010a, Lawson 2010b). Changes in testicular volume, histology, gonadosomatic indexes and other reproductive scores have all been used to document the reproductive cycle, the underlying mechanisms of testicular growth and regression are poorly understood.

*P. papilio* is a member of Family Periophthalmidae. It is indigenous member of brackish waters of estuarine and creeks. Its Importance lies in its contributions as food to man and baits for artisanal and offshore fisheries. It sells for as high as US$20/kg in Taiwan and Japan (Khaironizam and Norma-Rashid, 2002) and $15 equivalent in Nigeria. Related species in other parts of the world include *P. chrysospilos* and *Boleophthalmus boddaerti* in Singapore, *P. takita* was recently discovered in Australia (Jaafar and Larson, 2008). In Nigeria importance of *P. papilio* has attracted attentions of King and Udo (1996), Etim et al. (1996), Etim et al. (2002), Udo (2002), Lawson 2004a, Lawson 2004b, Lawson 2004c, Lawson 2004d) and Lawson (2011). Little information exists on the reproductive biology of the species especially concerning the males' testicular maturation and reproductive cycle. Aspects of its reproductive biology in Lagos lagoon, Nigeria was reviewed by Lawson (2010a) while the maturation and histological characteristics of ovaries was documented in Lawson (2010b), but made no histological analysis to propose maturity classes for testicular development of this species. However, there are several reviews on testes in some non related teleosts. These include Awaji and Hanyu (1987) on wild type Medaka, *Oryzias latipes*; Ratty et al. (1990) on gonad morphology, histology, and spermatogenesis in Albacore Tuna, *Thunnus alalunga*; in hybrid of Roach, *Rutilus rutilus* and bream, *Abramis brama* by Kopiejewska et al. (2004); Arockiaraj et al. (2004) on freshwater catfish, *Mystus montanus*; El-Greisy (2005) on Brushtooth lizard fish, *Saurida undosquamis*; Santos et al. (2006) on Characin, *Oligosarcus hepsetus* in Brazilian tropical reservoir; El-Halfawy et al. (2007) on Grey mullet, *Liza*

*ramada* in lake Timsah, Suez canal; and Chakraborty et al. (2007) reports on Sarpunti, *Puntius sarana* in Bangladesh.

Just as the ovary, the testicular maturity can be judged by visual observations by morphological and histological observations (Rath, 2000; Lawson 2010b). Histological study of this species though very strenuous is very essential especially in reproductive system. It is the most accurate method to determine the reproductive state of fish (West, 1990). The present study on testicular maturation of this species by histological investigation provides a basic knowledge of reproductive system in fish and will be a useful tool for further applications in other teleosts.

## 2.0 Materials and Methods

### 2.1 Collection of specimens:

A total of 779 individuals (comprising 16 immature and 763 mature males) of mudskipper, *Periophthalmus papilio* were caught from the mudflats of the mangrove swamps of Lagos lagoon (longitude: $3^{o}20'$-$3^{o}50'$W and latitude: $6^{o}24'$-$6^{o}36'$N) between July 2004 and July 2006. The fish were caught with non return valve traps. The diurnal collections were carried out with assistance of artisanal fishermen.

### 2.2 Laboratory procedures and data collections:

In the laboratory, collection of biometric data such as total length (TL) and body weight (BW) measurements were carried out, TL to the nearest 1 mm and BW to the nearest 0.1 g. The specimens were examined for abnormality or pathological changes and were cut opened through the ventral position. Sexes and gonad maturity stage were ascertained by naked eye examination of the gonads were confirmed under the light microscope. Testes were removed from the specimens, the paired testes were weighed (GW) to the nearest 0.01 g. The testes were fixed in Bouin's fluid. Sections were taken from the middle part of each testicular lobe, dehydrated in various percentages of alcohol, cleared in xylene, and impregnated in paraffin wax between 52-60 $^{o}$C melting points. They were embedded in paraffin wax and sectioned at 6 µm thick. The sections were stained in Eirlich haemotoxylin and Eosin (H&E) following Belelander and Ramaley (1979). Microscopic observations of the testes were done under binocular microscope that was mounted with camera and photographs taken. To determine the individual stage of sexual maturation, visual staging of testes was applied. The description of macroscopic criteria was developed by comparing the histological results with the photographic records of the gonads. Maturity stages were evaluated using scales from which each gonad was judged by visual analysis of external features. Sexual maturity of each specimen was classified according to macroscopic scales used in the IBTS (International Bottom Trawl Survey), BITS (Baltic International Trawl Survey), ICES, 1963 (International Council for Exploration of the Sea), (ICES, 1999) and recently, Bucholtz et al (2008) manual, and as well using a microscopic scale, based on histological analysis (Vitale et al., 2005). The microscopic criteria applied in the classification of testicular development were based on testes characteristics such as the formation of spermatogonia, spermatocytes, spermatids, and spermatozoa. This microscopic classification underlines the importance of the passage from endogenous to exogenous spermatocytes, which coincides with the beginning of milt production.

The length at which 50% of the fish population reached sexual maturity ($L_{50}$) was considered to be the length at first sexual maturity (Pitt, 1970).

The gonadosomatic index (GSI) of the fish was calculated by dividing the ovaries weight by the whole body weight and multiply by 100. Thus:

$$GSI = \frac{GW \times 100\%}{BW}$$

## 3.0. Results.

### 3.1 The structure of Testis

The testes were paired and joined posteriorly to form a Y-shaped structure. They appeared as a white multilobed filament and were flat and ribbon like at immature stage. At maturity they appeared creamy, soft, swollen, and multilobed. They lied ventrolaterally to the air bladder and were found in connection with a pair of accessory organ, which increased in size, as the fish grow in size or old.

### 3.2. Description of testicular maturity stages in *P. papilio*.

#### 3.2.1 Macroscopic characteristics of testes

In the present study, macroscopically the testes were classified into seven (7) stages of maturity which are discussed in Fig. 1.

#### 3.2.1.1. Immature or Stage I:

Specimens classified as immature were those that possessed gonads that were too small to be recognized as males or females. The gonads were too rudimentary in structure to be differentiated. They appeared too small occupying less than $1/10^{th}$ of the abdominal cavity. The naked eye examination did not reveal the presence of accessory sexual organs. Milt not released with gentle pressure.

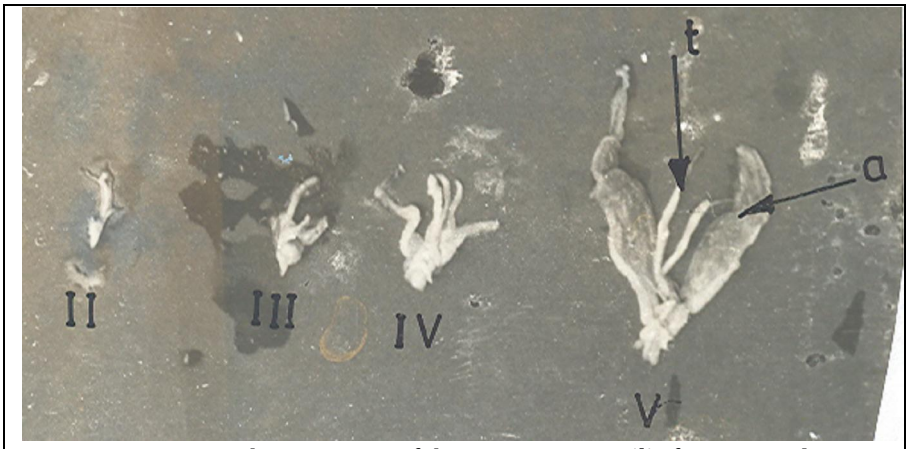



Fig. 1. Macroscopic characteristics of the testes in *P. papilio* from Lagos lagoon, Nigeria.
II, Immature and developing; III, Ripening; IV, Ripe; V, Ripe running; t, testis; a, accessory organ supporting testes.

**3.2.1.2. Immature and developing or Stage II:**

The testes were flattened and 1-2mm broad, whitish and lobed. They occupied 1% of the body cavity and the accessory sexual organs were rudimentarily visible and appeared small in size. No milt was released with gentle pressure.

**3.2.1.3. Ripening or Stage III:**

In early stage of ripening, the testes became fatter, off white and occupied 1/8$^{th}$ of the abdominal cavity. Blood vessels were visible through testis wall. Gonad length: width ratio was 2.8. In the late stage, the testes became firm and whiter and occupied 1/5$^{th}$ 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. Testes were large but milt not released with gentle pressure.

**3.2.1.4. Ripe or Stage IV:**

The testes were fully swollen and multilobed at this stage but did not occupy more than 1/4$^{th}$ of the body cavity. The color was creamy white. The accessory sexual organs grew past the testes. The testes were large with milt freely flowing with gentle pressure of the abdomen.

**3.2.1.5. Ripe running or Stage V:**

The testes were broadest 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.

**3.2.1.6. Spent or Stage VI:**

The testes reduced in size and sometimes very small, flaccid and walls were hard in texture. They were dark brown color and no blood vessel visible. Milts were absent or not released with gentle pressure, testis length: width ratio was 3.2 and gonad extended for 30% of the abdominal cavity.

**3.2.1.7. Recovering-spent or Stage VII:**

Dark patches visible through the testicular wall. The testes were small and about a third of length of body cavity or less and firmer than what obtained at spent stage and 3mm broad.

**3.2.2. Comparison of present study with other maturity scales.**

Table 1 describes the conversion of the scale developed in this study to the scales of Bucholtz et al (2008), and ICES (1963) and as well as the scales used for the BITS and IBTS surveys. Stages I, II, III, IV, V, VI VII and VIII represent degrees of maturation of the testes generated from the present study based on the stated methodology and as well as scales adapted from ICES, Bucholtz, BITS and IBTS. The ICES scale is commonly used in most laboratories. The ICES, BITS and IBTS and Bucholtz scales were similar except the addition of abnormal stage by Bucholtz covering a stage of reproductive malfunction (stage VIII). However, these scales were modified and simplified in this study for better understanding of the histology of this species. Common to all these scales were a recovering-spent stage which encompassed the final recovery of the spent gonad as well as the beginning of a new maturation cycle.

Table 1. Comparison of the present scale with other maturity scales currently in use.

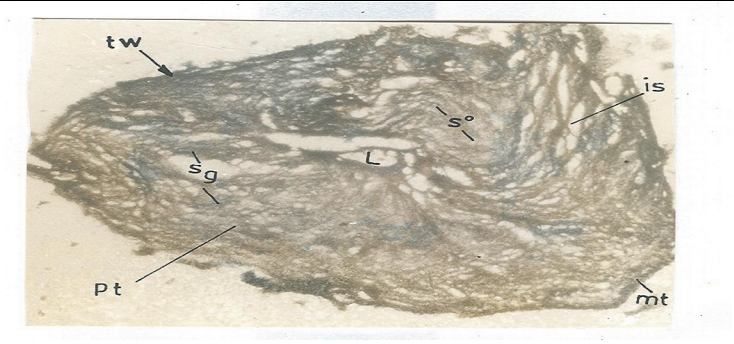
| Scale generated from the present study | | Current maturity scales in use | | | |
|---|---|---|---|---|---|
| Stage | Degree of maturation | Bucholtz et al 2008 | ICES | BITS | IBTS |
| I | Immature | I. Juvenile | I. Virgin | I. Virgin | I. Immature |
| II | Immature and developing | II. Early maturation | II. Virgin maturing<br>VII. Recovering- spent | II. Maturing | II. Maturing |
| III | Ripening | III. Mid maturation | III. Maturing | | |
| IV | Ripe | IV. Late maturation | IV. Maturing | | |
| V | Ripe running | V. Spawning capable | V. Maturing | | |
| VI | Spent | VI. Spawning | VI. Spawning | III. Spawning | III. Spawning |
| VII | Recovering-spent. | VII. Spent-recovery<br>VIII. Abnormal | VII. Spent | IV. Spent<br>V. Resting | IV. Spent |

ICES, International Council for Exploration of the sea; BITS, Baltic International Trawl Survey; IBTS, International Bottom Trawl survey.

### 3.2.3. Histological characteristics of testes:

The histological characteristics of different maturity stages in the testes of *P. papilio* are presented in Fig. 2.

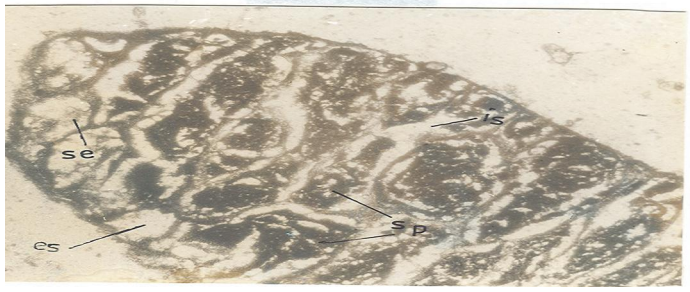
**Immature stage:** Microscopic examination showed no sexual differentiation. The cells associated with the gonads were rudimentarily developed and could not be differentiated. Hence the specimens were classified as immature. The sample size (N) for this group was 16 specimens.




**1000 µm**

a: Photomicrograph of T.S of a testis of *P. papilio* in Immature and developing stage

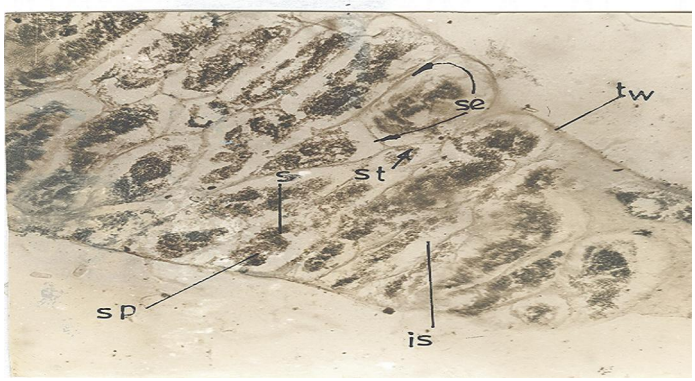
Testicular wall was thick with spermatogonia and primary spermatocytes pre-dominating the peritoneum (Fig. 2a). The mesothelium of the peritoneum was very thick. The stoma and interlobular septa were very conspicuous.
N=313.




**1000 µm**

c: Photomicrograph of T.S of a testis of *P. papilio* in ripe stage

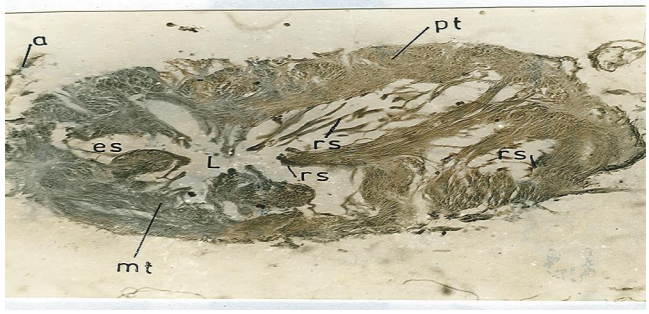
The primary and secondary spermatocytes were dominant while few spermatids and spermatozoa were represented. The testicular wall was 30µm. The testicular septa were well organized and distinct (Fig. 2c).
N=83.




**1000µm**

d: Photomicrograph of T.S of a testis of *P. papilio* in Ripe running stage.

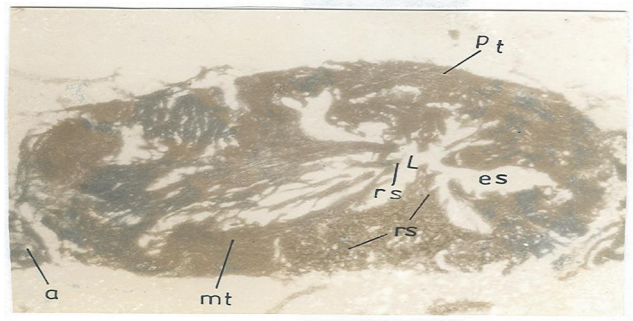
The lumen contained spermatozoa (Fig. 2d). The empty spaces in the lumen also contained spermatocytes and spermatozoa. Most of the spermatozoa migrated towards the periphery of the lobules and primary and secondary spermatocytes and the spermatids were found towards the interior. The testicular wall reached 30µm.
N=111.




**1000 µm**

e: Photomicrograph of T.S of a testis of *P. papilio* in spent stage

The testis had unfilled lumen with inactive or residual spermatozoa (Fig. 2e) The testicular wall reached 40µm. The accessory sexual organs well developed and longer than what obtained in ripe stage. The septa disappeared and mesothelium was thickest.
N=49.




**1000 µm**

f: Photomicrograph of T.S of a testis of *P. papilio* in recovering-spent stage.

A big cavity (lumen) was seen at the centre of the gonad and residual spermatozoa were present at the lumen (Fig. 2f). The mesothelium of the peritoneum thickened.
N=5.

Fig. 2a-f. Photomicrographs of Transverse section of testes of *P. papilo* in their different maturity stages in Lagos lagoon, Nigeria.
$s^o$, primary spermatocyte; is, interlobular septa; sg, spermagonium, pt, peritoneum; mt, mesothelium; L, lumen; es, empty space; st, spermatid; tw, testicular wall; lo=lobule, sp, spermatozoon; se, septum; s', secondary spermatocyte; s, subfollicular space; st, spermatid; tw, testicular wall; es, empty space; pt, peritoneum; a, accessory sex organ; mt, mesothelium; rs, residual spermatozoa; N, sample size.

Histological characteristics of the testes also revealed the process of spermatogenesis in *P. papilio*. The process occurred progressively during the annual reproductive cycle. This study showed five spermatogenic cells (Fig. 3) as follow:

**3.2.3.1. Spermatogonia:**

Primary and secondary spermatogonia were observed. The former were the largest spermatogenic cell, with clear cytoplasm, large nucleus, occurring isolated. The cysts or nests were absent. The latter were smaller and formed cysts that comprised a few cells.

**3.2.3.2 Primary spermatocytes:**

They possessed nuclei that were densely packed with chromatin material. They are product of repeated mitotic divisions of spermatogonia. The prominent nucleus was with filamentous chromatin.

**3.2.3.4. Spermatids:**

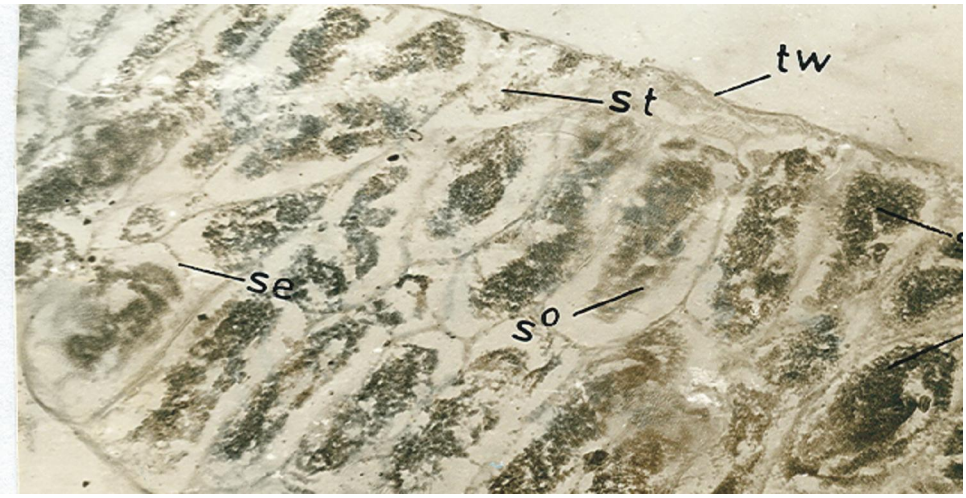
They were smaller in size than secondary spermatocytes. They are product of mitotic division or subsequent maturation of secondary spermatocytes. They were spherical and possessed dense nuclei.

**3.2.3.5. Spermatozoa:**

These are product of modifications or metamorphosis of spermatid cells. They are the smallest spermatogenic cells. They concentrated in the seminal lobules after breaking through the cyst wall. The sperms later acquired the ability to be mobile in the seminal fluid. This signifies the final product of the process of spermatogenesis.

**3.2..3.3. Secondary spermatocytes:**

They are in large nests that extended into the lobular lumen and characterized by homogenously stained nuclei. They are product of several meiotic divisions. The nucleus has condensed chromatin.




**1000µm**

Fig. 3: A cross section through mature testis of *P. papilio* showing some spermatogenic cells i.e. primary spermatocyte ($s^o$), secondary spermatocyte (s'), spermatid (st), and spermatozoon (s). tw, testicular wall; se, septum.

**3.3. Monthly distribution of male *P. papilio* in different maturity stages.**

The percentage distribution of different maturity stages in male *P. papilio* is given in Table 2. All stages of maturation were recorded in this study. Stage I testes were encountered in nine (9) of the 25 month(s) duration of study. Percentages varied from 1 in February 2006 and 6.52% in November 2004. Stage II testes occurred throughout the period of study. All male fish in May and June 2005, 2006 were in the immature and developing stage. Stage III, IV and V males showed a similar pattern of distribution occurring throughout the collection period except in May and June 2005, 2006. Stages VI and VII testes appeared in 9 and 4 months respectively. Few numbers were recorded.

**3.4. Size at different maturity stages**

Table 3 shows that all male fish (stage I testes) with measurements below 60 (48.87±9.92) mm TL and 2.0 (1.57±0.93) g BW were immature, while those (stages II-VII testes) longer than 65 and heavier than 2.2 were considered to be mature males. The mature fish ranged between the mean value of 97.03±15.24 and 139.98±19.35 mm TL weighing 10.53±4.86 to 30.69±10.99 g BW respectively.

The length at first sexual maturity for the male was 65 mm TL and 2.2 g BW.

### 3.5. Testicular reproductive cycle.

The reproductive cycle in male *P. papilio* was divided into three (3) phases (Fig. 4). The pre-spawning phase included immature, immature and developing and ripening stage testes. In this phase, blood capillaries were not very conspicuous; numerous spermatogonia were observed inside the small seminiferous lobules. Immature stage only occurred once in the life history of this fish.

The spawning phase, comprised testes in their ripe and ripe running stages. All stages of spermatogenesis were seen in their various lobules. The spermatogonia decreased in number due to intense spermatogenesis. Numerous primary and secondary spermatocytes were visible. The phase was characterized by primary spermatocytes that were smaller than spermatogonia. The secondary spermatocytes were smaller than the primary and were with chromatin. The blood capillaries were conspicuous; the seminiferous tubules were big and full of sperm masses.

The post-spawning phase consisted testes that were at spent and recovering-spent stages. Empty and collapsing seminiferous lobules were observed. Residual or unexpelled sperm were also seen. In addition recovering-spent testes showed developing spermatogonia inside the small seminiferous lobules.

Table 2. Monthly distribution of different maturity stages in *P. papilio* from Lagos lagoon, Nigeria.

| Year | Month | Total | Stage I | | Stage II | | Stage III | | Stage IV | | Stage V | | Stage VI | | Stage VII | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | no | % | no | % | no | % | no | % | no | % | no | % | no | % |
| 2004 | July | 8 | | | 1 | 12.5 | 1 | 12.5 | 5 | | 1 | 12.5 | | | | |
| | August | 10 | | | 1 | 10 | 3 | 30 | 4 | 40 | 2 | 20 | | | | |
| | September | 5 | | | 1 | 20 | 2 | 40 | 1 | 20 | 1 | 20 | | | | |
| | October | 10 | | | 6 | 60 | 3 | 30 | | | 1 | 10 | | | | |
| | November | 46 | 3 | 6.52 | 15 | 32.61 | 13 | 28.26 | 5 | 10.87 | 8 | 17.39 | 2 | 4.35 | | |
| | December | 39 | 2 | 5.13 | 13 | 33.33 | 5 | 12.82 | 3 | 7.69 | 3 | 7.69 | 13 | 33.33 | | |
| 2005 | January | 138 | 2 | 1.45 | 53 | 38.41 | 41 | 29.71 | 14 | 10.15 | 20 | 14.49 | 8 | 5.80 | | |
| | February | 51 | 2 | 3.92 | 17 | 33.33 | 18 | 35.29 | 4 | 7.84 | 9 | 17.65 | | | 1 | 1.96 |
| | March | 29 | | | 11 | 37.93 | 8 | 27.59 | 2 | 6.90 | 8 | 27.59 | | | | |
| | April | 6 | | | 1 | 16.67 | 1 | 16.67 | 3 | 50 | 1 | 16.67 | | | | |
| | May | 2 | | | 2 | 100 | | | | | | | | | | |
| | June | 4 | | | 4 | 100 | | | | | | | | | | |
| | July | 10 | | | 3 | 30 | 5 | 50 | 1 | 10 | 1 | 10 | | | | |
| | August | 12 | | | 5 | 41.67 | 4 | 33.33 | 1 | 8.33 | 8.33 | | 2 | 16.67 | | |
| | September | 6 | | | 4 | 66.67 | 2 | 33.33 | | | | | | | | |
| | October | 12 | | | 5 | 41.67 | 2 | 16.67 | | | 2 | 16.67 | 3 | 25 | | |
| | November | 86 | 1 | 1.16 | 24 | 27.91 | 29 | 33.72 | 9 | 10.47 | 10 | 11.63 | 12 | 13.95 | 1 | 1.16 |
| | December | 73 | 2 | 2.74 | 42 | 57.53 | 15 | 20.55 | 5 | 6.84 | 7 | 9.59 | 2 | 2.74 | | |
| 2006 | January | 72 | 2 | 2.78 | 40 | 55.56 | 17 | 23.61 | 3 | 4.17 | 9 | 12.5 | 1 | 1.39 | | |
| | February | 100 | 1 | 1.0 | 38 | 38.0 | 28 | 28.0 | 12 | 12.0 | 14 | 14.0 | 6 | 6.0 | 1 | 1.0 |
| | March | 40 | 1 | 2.5 | 14 | 35.0 | 14 | 35.0 | 3 | 7.5 | 7 | 17.5 | | | 1 | 2.5 |
| | April | 20 | | | 7 | 35.0 | 4 | 20.0 | 4 | 20.0 | 5 | 25.0 | | | | |
| | May | 4 | | | 1 | 25.0 | 1 | 25.0 | 1 | 25.0 | 1 | 25.0 | | | | |
| | June | 3 | | | 3 | 100 | | | | | | | | | | |
| | July | 10 | | | 2 | 20.0 | 3 | 30.0 | 3 | 30.0 | 2 | 20.0 | | | | |
| Total | | 796 | 16 | | 313 | | 219 | | 83 | | 112 | | 49 | | 4 | |

**Table 3. Fish size at different maturity stages in *P. papilio*.**

| Maturity stage | Range (total length in mm) | | | Range (body weight in g) | | |
|---|---|---|---|---|---|---|
| | min. | max. | Mean±SE | Min | max | Mean±SE |
| I | 37 | 60 | 48.87±9.92 | 1.5 | 2.0 | 1.57±0.93 |
| II | 65 | 145 | 97.03±15.24 | 2.2 | 30.9 | 10.53±4.86 |
| III | 76 | 163 | 116.68±17.73 | 5.6 | 43 | 18.33±8.68 |
| IV | 85 | 180 | 127.03±22.49 | 6.8 | 60.9 | 23.30±12.54 |
| V | 90 | 168 | 139.98±19.35 | 7.4 | 52 | 30.69±10.99 |
| VI | 100 | 165 | 135.39±18.46 | 11.2 | 45.6 | 26.50±9.42 |
| VII | 114 | 132 | 124.2±7.36 | 14.4 | 28.3 | 21.4±5.13 |

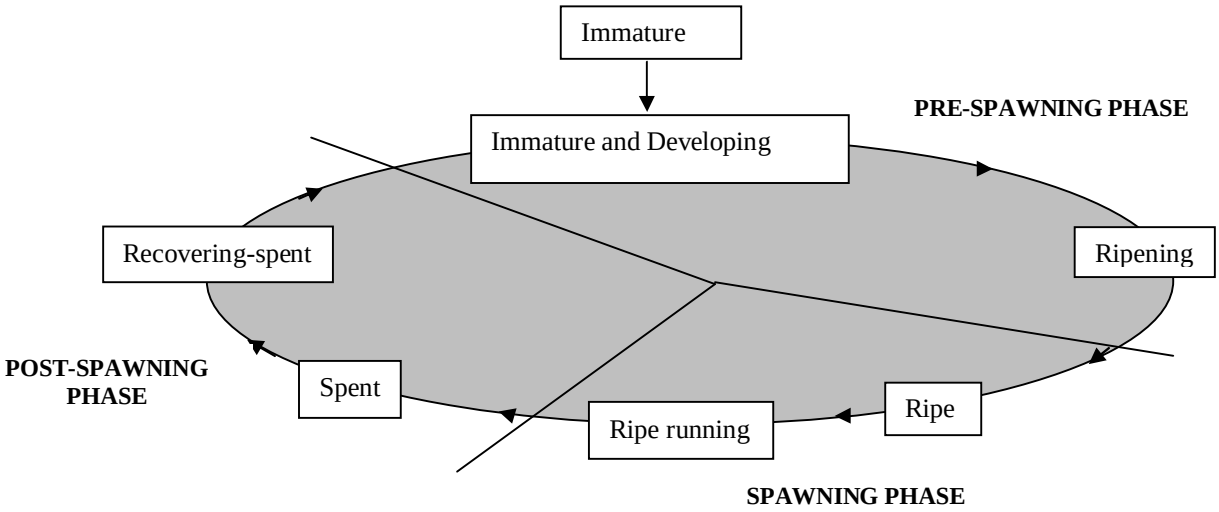



Fig. 4. A schematic diagram of reproductive cycle in male *P. papilio* from Lagos lagoon, Nigeria.

### 3.6. Frequency distribution of Reproductive phases

The histograms of frequency distribution of reproductive phases of this species are presented in Fig. 5. Of the seven (7) maturity stages and three (3) maturations phases encountered in the study, testes in the stage II were the most abundant constituting 39.32% of the population. The least was stage VII testes that contributed 0.5%. The pre-spawners were more in number than the spawning or post-spawning fish constituting 68.84, 24.50 and 6.66% of the catch respectively.

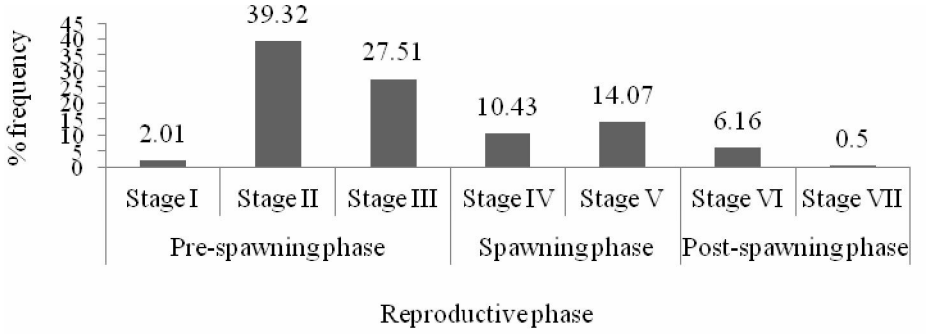



Fig. 5. Reproductive phases in testes of *P. papilio* from Lagos lagoon , Nigeria.

### 3.7. Gonadosomatic index (GSI) of *P. papilio.*

The monthly GSI values in male *P. papilio* from Lagos lagoon are presented in Table 4. The values varied between 0.01 and 0.48 % in August and September 2004 respectively. The overall mean GSI value was 0.132±0.17%.

The polygons of monthly mean GSI (Fig. 6) revealed significant differences ($P<0.05$) among the different stages of maturity in the fish. The lowest value of 0.02±0.002 % was recorded in August 2005 and 0.865±0.12% in January 2005. The figure showed different peaks of mean GSI in July (0.23±0.016) and September (0.30±0.13%) 2004; May (0.198±0.004) and October (0.097±0.009%) 2005; and January (0.865±0.12), April (0.122±0.009) and July (0.145±0.016%) 2006.

Table 4. The GSI values for male *P. papilio* in Lagos lagoon, Nigeria

| Year | Month | Range | |
|---|---|---|---|
| | | Minimum | Maximum |
| 2004 | July | 0.16 | 0.28 |
| | August | 0.01 | 0.06 |
| | September | 0.11 | 0.48 |
| | October | 0.08 | 0.10 |
| | November | 0.04 | 0.32 |
| | December | 0.05 | 0.12 |
| 2005 | January | 0.034 | 0.28 |
| | February | 0.015 | 0.217 |
| | March | 0.039 | 0.301 |
| | April | 0.104 | 0.19 |
| | May | 0.09 | 0.28 |
| | June | 0.05 | 0.09 |
| | July | 0.041 | 0.076 |
| | August | 0.01 | 0.045 |
| | September | 0.02 | 0.081 |
| | October | 0.084 | 0.11 |
| | November | 0.015 | 0.102 |
| | December | 0.017 | 0.076 |
| 2006 | January | 0.08 | 0.189 |
| | February | 0.03 | 0.25 |
| | March | 0.03 | 0.26 |
| | April | 0.07 | 0.25 |
| | May | 0.05 | 0.09 |
| | June | 0.02 | 0.05 |
| | July | 0.03 | 0.09 |

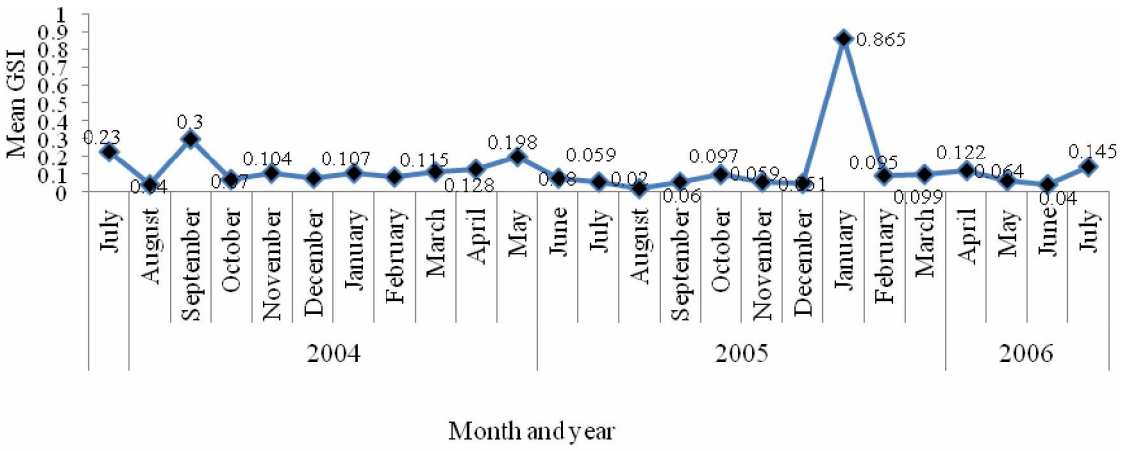



Fig. 6. Monthly mean GSI in testes of *P. papilio* from Lagos lagoon, Nigeria.

**4.0. Discussion.**

In the present study seven stages of testicular maturation were developed. They are immature (stage I), immature and developing (II), ripening (III), ripe (IV), ripe running (V), spent (VI) and recovering-spent (VII) stages. These were classified into 3 reproductive phases. Stages I-III were testes in pre-spawning phase, IV and V were spawners while VI and VII were classified as post spawning testes. This result was additional data and an improvement over the scales that

were generated by ICES (1963, ICES, 1999), BITS, IBTS and Bucholtz et al (2008). A scale generated from the present study was a modification of the ICES, BIT, and IBTS scales that were used in the current study (Table 1). The general pattern of histological development of the testes of the present study conforms to that of the most teleosts. A 4-stage maturity scale was generated by 1BTS, 5 by BITS, 7 by ICES and 8 by Bucholtz et al (2008) for Herrings and Cods. These scales were reportedly applied in histological study of many teleosts. The maturity stages are hardly discernible by the naked eye and consequently the most susceptible to misclassification.

The histological development of testes showed that cells in immature testes were not discernible to be differentiated as males or females. Those at ripening stage showed active spermatogenesis, while those at ripe and ripe running stages indicated swollen lobules with sperm that were typical of fish in their breeding period. Testes at spent stage presented lobules with residual spermatozoa. Fish in the recovering-spent stage showed testes with spermatogonia and spermatocytes along the wall of seminal lobules, while the lumens of the lobule were with spermatids.

The immature stage can only occur once. Immature and developing signals the entry of the fish into gonadotropin dependent spermatogenesis and gonadal growth. In ripening stage, testes begin to develop not spawn soon. Ripe and ripe running testes indicate that the spermatozoa in lumen are sufficiently advanced and capable of spawning. Spent stage indicates a regressing phase when there was cessation of spawning or completion of spawning season in their burrows. Those in their recovering-spent stage are sexually mature but reproductively inactive. These reports supported and were added data to the study conducted by Brown-Peterson et al (2007). The histological criteria identifying stages of maturity may vary for different species and may not always occur sequentially, but each stage is conceptually universal.

Development of sperm or milt in fish according to Shein et al (2004) passes through multiplication stage, growth and maturation stages. The testes of *P. papilo* consisted of seminiferous tubules, spermatogonium, spermatocyte, spermatids and spermatozoon as were observed in Fig 2 and 3. Their presence was an indication that the gamete or sperm had gone through process of maturation (spermatogenesis). Spermatogonium is a primordial male germ cell that may divide by mitosis to produce primary spermatocyte. The spermatocyte undergoes two meiotic divisions to form four spermatids, which further divisions give rise to spermatozoa.

The length at first sexual maturity of male *P. papilio* in Lagos lagoon was 65 mm TL. Teleosts attain sexual maturity at various ages depending on the species, latitude, water temperature, salinity. The age, at which fish living in a water body under natural environmental conditions (in regard to age and season) attain maturity depends on the latitude. The environmental factors such as temperature, photoperiod, nutrient supply, dissolved oxygen, diseases or parasites are well known to influence reproductive maturity in fish (Cambray, 1994; Joy et al 1999). But the mechanism of action of various environmental factors as well as the sites of their action remains to be determined at the cellular and molecular levels (Lawson 2010b).

The fish burrowed and spawned in the mud flats, this was responsible for fewer populations of the spawners and post spawning fish. Fish close to spawning phase enter the spawning nests and stayed there for some while even at spent stage. This may be reason for the higher percentage of pre-spawners (68.84%) than either spawning (24.50%) or post-spawning (6.66%) fish recorded in the present study. The same trend was reported by Lawson (2010b) in female *P. papilio* in Lagos lagoon. Nest spawning behaviour was reported in *B. pectinirostris*, *P. cantonensis* ,and *P. modestus* by Uchida (1931); and Dotsu and Matob (1977) in Ariake sound and Washio et al (1993) in Midori River, Kumamoto prefecture in Japan. The maturation following their migration to the spawning nest could also responsible for their inability to be collected with gears and traps.

In the present study, there was variation in the monthly GSI values, this according to Washio et al (1993) reports on mudskipper species, *B. pectinirostris* is closely related to the annual changes in reproduction. The highest GSI values correspond to when the testes were at ripe and ripe running stages (spawning phase) while the lowest GSI values indicate totally spent stage. The recovering-spent, immature and developing and ripening stages showed slightly higher mean GSI values than the spent stage. The different peaks of mean GSI values that were observed in the months of July, September, May, October, January and April presumed the fish as a multiple and synchronous spawner in Lagos lagoon. The species spawn several times within a spawning season. The female was reported as a multiple spawner in Lagos lagoon by Lawson (2010b). This probably contributes to reproductive success of the species in Lagos lagoon.

In Lagos lagoon, between 0.01 and 0.48% of the body mass was converted to gonad development by the fish. The GSI varied with species, sex, seasons and availability of food and these were in conformity with reports from other teleosts (Lawson and Aguda 2010; Lawson and Jimoh, 2010; Lawson et al 2010; Lawson, 2011) in some Nigerian waters. The GSI had been used to describe the development of gonads in Pike, *Esox lucius* by Danilenko (1983). It has been widely used as indicator of the fish spawning period, but its use in

reproductive biology studies is more suitable when associated with other reproduction indicators such as macroscopic and histological techniques. This is very important in males since differences in size (length and weight) according to Chaves (1991) are less conspicuous than in females. GSI increases progressively with increases in the percentages of ripe individuals towards the spawning seasons (Mohamed, 2010).

In *P. papilio* GSI values increased from recovering-spent to ripening stage, reaching a peak in ripe or ripe running stage, followed by a decreasing trend to the spent stage. This pattern is expected in teleosts. 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 Assem 2003; Honji et al. 2006).

Therefore, the study has provided information on the testicular maturation process and reproductive cycle of mudskipper, *P. papilio* in Lagos lagoon, Nigeria. The study also contribute baseline data towards management ecology, conservation and biological studies of this and other commercially valued fish in Lagos lagoon complex.

There is an on going research work of the ultrastructural characteristics of the gonads in this species using a transmission electron microscope. The reports of the study will be reported in the next paper.

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