Maturation and Histological characteristics of ovaries in Mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria.

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Abstract: Maturation and histological characteristics of female gonads in mudskipper, *Periophthalmus papilio* from Lagos lagoon, Nigeria were investigated between July 2004 and July 2006. This species is found in abundance in the mudflats of the mangrove swamps of Lagos lagoon where it forms part of its fisheries. Its importance lies on its availability as food for man and as baits for both artisanal and offshore fisheries. Diurnal collections were made with non return valve traps. Biometric data were recorded and sexes separated. Ovaries were carefully removed from 1390 individual specimens that were with no abnormalities or pathological changes. The histological structure of the ovaries was based on a temporal scale after intensive sampling. The ovaries were observed macroscopically and processed by standard histological technique. ICES, BITTS and IBTS scales and Bucholtz manuals were employed in the classifications of its maturity and gonadal stages. Seven stages of maturity which included: immature (stage I), immature and developing (stage II), ripening (stage III), ripe (stage IV), ripe running (Stage V), spent (stage VI) and recovering-spent (stage VII) were observed among the specimens. These constituted 1.15, 47.99, 15.32, 9.86, 19.50, 4.68 and 1.51% of the specimens examined in the study respectively. The pre-spawning phase was represented by stages I, II and III; the spawning by IV and V; and post-spawning by VI and VII. Histological development of the species indicated six (6) developmental stages of oocytes development viz: oogonium, primary oocyte, primary, secondary, and tertiary vitellogenic and hyaline oocytes. Specimens were found with oocytes which had developed over the migratory nucleus stage, indicating maturation can still proceed in the fish on the mudflats before migrating to spawning nests in the burrows. Stages V and VI ovaries contained all stages of oocyte. The GSI of the species increased at initial phase and then became stable at the later period. The species was a multiple and synchronous spawner, spawning in February, March, and October. The mean GSI varied from 1.03± 0.09% in May to 8.40±1.67% in February 2006. Less than 8.40±1.67% of the body biomass was converted by the species to development of ovaries. The minimum size of spawning females was 110 mm TL. Therefore, this study provides the necessary information on maturation and histological development of oocytes as an appropriate strategy for optimum utilization and conservation of this commercially valued fish species in Lagos lagoon, Nigeria.


Key words: Chromatin, zona radiata interna, externa, maturation, nucleolar, vitellogenic.

1. Introduction

Mudskipper, *Periophthalmus papilio* (Bloch and Schneider 1801) is a member of the family Periophthalmidae. It is the only reported species of the family in the Gulf of Guinea, which includes Lagos lagoon in Nigeria (FAO, 1990; Lawson, 1998), where it has been reported in large number (Etim et al. 1996; King and Udo, 1996; Udo 2002). Irvine (1947) grouped *Periophthalmus* into indigenous or permanent element of the brackish waters of estuaries and lagoons. Other related species found in other parts of the world include: *P. chrysospilos* 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. Importance of this fish lies on its commercial value as food especially in Niger Delta region and as bait in artisanal and offshore fisheries. It is reported to cost as high as $20/kg in Taiwan and Japan (Khaironizam and Norma Rashid 2002). Reviews on the *P. papilio* include that of King and Udo (1996) on its length-weight relationships; Etim et al (1996) gave a report on its population dynamics in Eastern Nigeria; and Lawson (1998) documented the aspects of its bioecology; its distribution, age determination, and growth patterns (Lawson, 2004a); its salinity tolerance and preference (Lawson, 2004b); and its blood osmolality contents (Lawson, 2004c). Aspects of its food and feeding habits (Lawson, 2004d); length-weight relationships and fecundity estimates (Lawson, 2011) were also investigated in Lagos lagoon, Nigeria. Several reviews on the maturation, histological and ultrastructural characteristics of non related species include that of Marcus (1982) on

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 female fish (West, 1990). Therefore, the study on histology of ovaries of fish will provide a basic knowledge of reproductive system of fish and will be a useful tool for further applications in other species. This study has sought to investigate maturation and characterize the histology and ultrastructure of the ovary in mudskipper, *P. papilio* from the mangrove swamps of Lagos Lagoon, Nigeria.

2.0 Materials and Methods

2.1 Collection of specimens:

1390 female individuals of mudskipper, *Periophthalmus papilio* were caught from the mudflats of Lagos lagoon (longitude: 3°20′-3°50″W and latitude: 6°24′-6°36″N) between July 2004 and July 2006. The diurnal collections were carried out with non return valve traps. Services of artisanal fishermen were employed.

2.2 Laboratory procedures and data collections:

In the laboratory, collections of biometric data such as sex, 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 stages were ascertained by naked eye examination of the gonads and were confirmed under the light microscope. Ovaries were removed from the specimens considered to be females, the paired ovaries were weighed (GW) to the nearest 0.1 g. The ovaries were fixed in Bouin’s fluid. Sections were taken from the middle part of each ovarian lobe, dehydrated in alcohol, cleared in xylene, and impregnated in paraffin wax between 52–60 °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 ovaries were done under binocular microscope that was mounted with camera and photographs taken.

To determine the individual stage of sexual maturation, visual staging of reproductive organs was applied. The description of macroscopic criteria was developed by comparing the histological results with the photographic records of the ovaries. 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 (International Council for Exploration of the Sea of 1963, 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 ovarian development were based on oocyte characteristics such as the formation of cortical alveoli, degree of yolk accumulation and nuclear migration. This microscopic classification underlines the importance of the passage from endogenous to exogenous vitellogenesis, which coincides with the beginning of yolk production in the oocytes.

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

\[ \text{GSI} = \frac{\text{GW} \times 100\%}{\text{BW}} \]
3.0 Results

3.1 The structure of ovary in *P. papilio*.

The morphology of ovaries in different developmental stages is presented in Figure 1. Ovary of *P. papilio* was observed to be a paired, elongated bodies situated in the posterior half of the body cavity and suspended from the body wall by the mesovarium. Anteriorly, the two lobes were free but posteriorly they bent downwards and inwardly to form a short oviduct leading to the genital pore. The length, width, and colour of ovaries were seen changing as maturity progressed due heavy vascularization. The colour turned yellow on maturation and reddish when the fish were ready to spawn (in stages IV and V). Stage I ovaries were not represented because they were not discernible enough to be classified as males or females.

3.2. Macroscopic characteristics of ovaries.

Macroscopically ovaries in *P. papilio* were classified into seven (7) developmental stages (Table 1). The stages were classified as Immature (Stage I), Immature and Developing (Stage II), Ripening (Stage III), Ripe (Stage IV), Ripe running (Stage V), Spent (Stage VI), and Recovering-spent (Stage VII).

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Degree of maturation</th>
<th>External or macroscopic appearance of females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Immature</td>
<td>The external examination did not show sexual differentiation, the gonads were rudimentarily developed and could not be differentiated as males or females. Hence the specimens were classified as immature. 16 specimens were observed as immature.</td>
</tr>
<tr>
<td>Stage II</td>
<td>Immature and developing</td>
<td>The ovaries were small, rounded with a rough surface and soft texture. They were pinkish in colour, translucent with blood vessels forming internally, and occupying between 1/8th (12.5%) and 1/4th (25.0%) of the length of the abdominal cavity. None of the oocytes were visible.</td>
</tr>
<tr>
<td>Stage III</td>
<td>Ripening</td>
<td>The ovaries were swollen and lobed. A heavy network of vessels appeared externally on the surface of the ovarian wall. Yellowish oocytes were visible to naked eye through the ovarian wall. The gonad extended for about 60 – 70% of the abdominal cavity.</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Ripe</td>
<td>Ovaries at this stage were almost filling the body cavity occupying 80 –90% of abdominal cavity. They were orange yellowish in colour. 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.</td>
</tr>
<tr>
<td>Stage V</td>
<td>Ripe running</td>
<td>The 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.</td>
</tr>
<tr>
<td>Stage VI</td>
<td>Spent</td>
<td>The red ovaries were flaccid and vascularized with reduced size, the ovarian wall was tough and smooth with no granulation. The residual eggs were visible through the flabby wall. The ovary length: width ratio was 4.5 and the gonads occupied 50% of the abdominal cavity. There were large numbers of surface blood vessels.</td>
</tr>
<tr>
<td>Stage VII</td>
<td>Recovering-spent</td>
<td>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 were visible through the ovary wall.</td>
</tr>
</tbody>
</table>

3.3. Comparison of present study with other maturity scales.

Table 2 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. The ICES scale is commonly used in most laboratories, the BITS and IBTS scales were similar. Also similar were Bucholtz et al (2008) and ICES (1963) scales except the addition of abnormal stage in former covering a stage of reproductive malfunction (stage VII). However, these scales were modified and simplified in this study for better understanding of the histology of this species and other teleosts. 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.

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3.4. Histological characteristics of Ovaries in P. Papilio.

The histological characteristics of the ovaries of this species in their different developmental stages are represented with photomicrographs in Figure 2A-F.

3.4.1 Immature and developing stage:

Histological appearance (Figure 2A) of the ovary was characterized by the presence of many oocytes between 0.025 and 0.05 mm. The larger oocytes were seen with cytoplasmic vacuoles and were irregularly shaped but few were rounded. The thickness of the ovarian wall was 50 µm and folded. Empty follicles and space were visible.

667 specimens belong to this category.

3.4.2 Ripening:

The histological observation of the ovaries at this stage showed that many oocytes between 0.1 and 0.2 mm were present. Larger oocytes with cytoplasmic vacuoles were very few and had small yolk droplets (Figure 2B). 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. N=213.

3.4.3. Ripe:

The histological observation of the gonads showed that the secondary and tertiary vitellogenic oocytes dominated the gonad with very few primary oocytes (Figure 2C). The theca externa were prominent. The hyaline oocytes were present but
usually collapsed by histological processing. Ovary wall was 90 µm thick; many oocytes were between 0.2 to 0.5 mm in diameter and usually 0.35 mm in size. Many oocytes were at stages II and III. There were blood vessels internally but some of the yolky oocytes were atretic. N=137.

3.4.4. Ripe running:
Oocytes looked exactly like those in the ripe stage and were laid singly with space (septa) in between as shown in Figure 2D; most of the oocytes were in their tertiary vitellogenic stage. N=271.

3.4.5. Spent:
A few atretic residual oocytes were seen, the invasion of oocytes by follicular cells was noted (Figure 2E). High level of oocyte atresia was noted. There was disorganization of septum, no empty follicular coat. The ovarian wall was 300 um thick while the lumen contained debris of the residual cells N=65.

3.4.6. Recovering and resting:
The residual atretic oocytes were present but the septum was not very organized (Figure 2F). Reorganization of ovigerous lamellae started. A few reabsorbing oocytes were also present. N=21.

The vascularized and ripe stage oocytes showing different developmental characteristics are present in Figure 3. The six (6) oocyte developmental stages in this study included: oogonium, primary oocyte, primary, secondary, tertiary vitellogenic and hyaline oocytes.
Figure 2. Photomicrographs of ovaries in their various maturation stages in *P. papilio* from Lagos lagoon, Nigeria. A: A section through an ovary in immature and developing; B: An ovary in ripening stage; C: A section through a ripe stage ovary; D: An ovary showing tertiary vitellogenic or ripe oocytes in ripe running stage; E: A section through an ovary in spent stage; F: An ovary in recovering-spent stage.

o, oogonium; po, primary oocyte; es, empty space; ef, empty follicle; ow, thick ovarian wall; s°, secondary oocyte; no, nucleolus; y, yolk; pv, primary vitellogenic oocyte; tv, tertiary vitellogenic oocyte; ga, gap between ovigerous fold; of, ovigerous fold; bv, blood vessel; ha, haline oocyte; ct, connective tissue; se, septum; ao, atretic oocyte; r, rupture ovarian wall.

Figure 3. The vascularized and ripe stage oocytes showing different developmental characteristics. s°, secondary oocyte, po, primary oocyte; pv, primary vitellogenic oocyte; es, empty space; ow, thick ovarian wall; tv, tertiary vitellogenic oocyte; ga, gap between ovigerous fold; o, oogonium; of, ovigerous fold; bv, blood vessel; ha, haline oocyte; y, yolk; ct, connective tissue; cm, chromatin; fe, follicular epithelium layer; zri, zona radiata interna; zre, zonal radiata externa; n, nucleus.
3.5. Reproductive cycle and maturity stages

In the present study seven stages of maturity were developed and validated (Figure 4). These stages were grouped into three phases as presented in Figure 4. The phases were (a) Pre-spawning phase which included stages I-III ovaries; (b) Spawning phase, the stages IV and V; and (c) post-spawning phase which were stages VI and VII. The reproductive cycle of P. papilio in Lagos lagoon started from stage I and ended at stage VII then back to stage II, or from the pre-spawning through spawning to post spawning, back to pre-spawning phase in cyclic manner.

![Reproductive cycle and maturity stage in P. papilio from Lagos lagoon, Nigeria.](image)

3.6. Distributions of maturity stages and phases in P. papilio.

Of the seven (7) maturity stages and three (3) maturations phases (Figure 5) encountered in the study, the least dominant group was stage I and stage II fish were the most abundant constituting 1.15 and 47.99% of the population respectively. The pre-spawning, spawning and post spawning phases were 64.46, 29.36 and 6.19% respectively. The pre-spawners were more in number than the spawning or post-spawning fish.

3.7. Gonadosomatic index of P. papilio

Monthly changes in GSI of the fish were presented in Figure 6. GSI were high in August and October 2004. The lowest GSI value of 1.03±0.09% was recorded in May and was at the peak (8.4±1.67%) in February, 2005. In 2005, GSI began increasing from January (6.36±1.23%), and these values represented the changes similar to those of 2006, although the GSI value in February 2005 that was high was significantly lower in 2004 and 2006 than those in 2005 (t-test, P<0.05).
4. Discussion

In this study, seven stages of gonadal development were observed in mudskipper, *Periophthalmus papilio* (Table 1). The stages were immature, immature and developing, ripening, ripe, ripe running, spent and recovering-spent. Immature fish were those that were unable to be differentiated both micro and macroscopically as males or females. Most of the specimens were at pre-spawning phase. Fewer fish populations at the spawning and post spawning phases was an indication that the fish had not migrated away from their spawning nests in their burrows.

The macroscopic characters and gonad differentiations occurred as maturation progress from a stage to next. Vascularisation and identification also increased with progression in size and maturity. Immature, immature and developing and ripening stage were categorized as pre spawning period, i.e a period when the fish were virgin, or maturing or were in their early or mid or late maturation phase. 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 2). The general pattern of histological development of the ovaries of the present study conforms to that of the most teleosts (El-Gharabawy, 1996; Assem, 2000 and 2003). A 4-stage maturity scale was generated by IBTS, 5 by BITLS, 7 by ICES and 8 by Bucholts et al 2008 for Herring and Cod. 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 six (6) developmental stages (Figure 3): oogonium, primary oocyte, primary, secondary, tertiary vitellogenic and hyaline oocytes were represented the various stages of the oocyte growth and development in P. papilio. This also confirmed progressive process in stages of formation, growth or development of eggs (oogenesis). These developmental stages were well documented by Gardner and Snustad (1984). Oocyte is the mother cell, the cell that undergoes two meiotic divisions to form the egg cell. The primary oocyte occurs before the completion of the first meiotic division; second oocyte, after the completion of the first meiotic division. Oogonium is a germ cell of the female before meiosis begins. Oogenesis in fish according to Jackson and Sullivan (1995) is accompanied by conspicuous cellular, biochemical, molecular and endocrinological changes.

The present study confirmed that the maturation period in P. papilio was characterized by appearance of isolated follicular epithelial cell around the oocyte and formation of yolk nuclei. The yolk nuclei appear first as a small spherical corpuscle in close adherence to one side of the nucleus and then migrate to the periphery of the cytoplasm, where it finally disintegrates and disappears. This was in agreement with reports of Mohamed (2010) on M. merluccius. Herrera et al (1988) pointed out that the follicular epithelial cells are considered as a good proof for synthesis of sexual steroids in fish.

The vacuolization period may be characterized by the presence of marginal vacuoles and by the fact that the oocyte wall consisted of zona radiata coated with follicular epithelial layer (Mohamed, 2010). Grant (1990) characterized the vacuolization stage by cortical alveoli formation.

The yolk deposition as presented in Figure 3 was a period characterized by the presence of yolk granules in the periphery of the oocyte cytoplasm. The yolk deposition in the oocytes in P. papilio showed the same picture described by many authors for some fishes (El-Gharabawy, 1996; Assem, 2000 and 2003) most of cytoplasm is filled with yolk granules of various sizes.

Examination of the ovaries of this species in this study showed presence of oocytes at different stages of development. This is an indication that the fish has prolonged and fractional spawning season. Therefore, the fish may spawn more than once along the spawning period. This was supported by Salem et al (1994) for Mugil seheli, El-Greisy (2000) for Diplodus sargus, Honjii et al (2006) for Merluccius hubbi; Garcia Diaz et al (2006) for Serranus atricauda; and Mohamed (2010) for Merluccius merluccius.

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 more south a water body in the northern hemisphere is found, the earlier the fish mature. The environmental factors such as temperature, photoperiod, nutrient supply, dissolved oxygen, diseases or parasites) are well known to influence reproductive maturity and oogenesis 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.

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 large number of pre-spawners than either spawning or post-spawning fish as reported in the present study. Nest spawning behavior was reported in B. pectinirostris, P. cantonensis and P. modestus by Uchida (1932); and Dotsu and Matoba (1977) in Ariake sound and Washio et al (1991) 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 traps.

GSI values were higher in 2005 than in 2004 or 2006 (Figure 5), the difference according to Washio et al (1991) reports on mudskipper species, B. pectinirostris is closely related to the annual changes in reproduction. The GSI had been used to describe the development of gonads in Pike, Esox lucius by Danilenko (1983). However, determination of reproductive maturity using only the GSI is not enough because the structures within the ovary, such as oocytes at different stages, interstitial tissues with accumulation of yolk materials, can not be interpreted by weight (Srijunngam and Wattanasirmkit, 2001). GSI increases progressively...

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with increases in the percentages of 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 and 2003; Honji et al., 2006). High values of GSI for the months of October 2004 (4.89±1.06%); February 2005 (8.4±1.67%); and March 2005 (7.53±2.56%) demonstrated that the species was a multiple spawner and spawned several times within a spawning period. Less than 8.40±1.67% of the body mass was converted to gonad development in the fish. 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. Therefore, the study provides information on the maturation process and histological characteristics in a species of mudskipper, *P. papilio*, an economically valued fish from Lagos lagoon, Nigeria. 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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