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Cover image: A seagrass meadow (*Thalassia hemprichii*) at Inhaca Island, Mozambique (© José Paula, 2017)

Reproductive biology and body condition of exploited populations of Emperor Angelfish, *Pomacanthus imperator* (Bloch, 1787) along the Kenyan Coast

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Abstract

A substantial proportion (in terms of value and quantity) of the *Pomacanthus imperator* catch on the Kenyan coast are traded, and the species is also harvested as food in the artisanal fishery. However information on their reproductive biology is scanty. The overall sex ratio differed significantly from 1:1 according to chi-square test ($p<0.05$). Sizes at maturity (L_{50}) were estimated to be 25cm and 28cm TL for females and males respectively. Fecundity was estimated to be in the range of 17,790-266,472 with a Mean ±SE of 79,353±11,747, and was linearly related to total length and ovary weight. March-April was the main period of reproductive activity based on gonad somatic indices and monthly proportion of mature individuals. The LWR indicated isometric growth both in males and females of *P. imperator,* as the allometric coefficient *b* values were not significantly different from the expected isometric value of 3 (Student's t-test; $p=0.12$). Relative condition factor did not vary significantly between the months sampled. The reproductive parameters obtained from this study provide some baseline information for management of this species which has proven to be highly vulnerable to depletion due to overfishing.

Keywords: Emperor Angelfish, *Pomacanthus imperator*, reproduction, body condition, Kenya.

Introduction

Angelfishes of the genus *Pomacanthus* are among the most highly prized aquarium reef fishes traded worldwide, and are a favorite for divers and aquarists alike (Thresher, 1982; Nelson, 2006). The emperor angelfish, *P. imperator* Bloch, 1787, is among the most spectacularly colored and broadly recognized species of coral reef-associated fishes. Globally, the family comprises about 25% in volume of the estimated 1472 aquarium species traded annually (Sadovy, 1992; Wabnitz *et. al*., 2003). The genera *Pomacanthus* belong to the group of larger angelfishes (Hiroshi, 1994).

In Kenya, the genus forms a substantial proportion of the aquarium fish trade in terms of value and abundance (Okemwa *et al*., 2016). A large abundance of *Pomacanthus* angelfishes has been reported in the western Indo-Pacific (Fricke, 1999; Pyle, 2001) from the Red sea and East Africa, to the Hawaiian, Line and Tuamoto Islands. A second main area of abundance has also been recognized from Southern Japan south to the Great Barrier reef, the Austral Islands and New Caledonia (Froese and Pauly, 2006).

The exploitation patterns in the aquarium fisheries in Kenya focus on specific fishing grounds and particular species (Okemwa *et al*., 2016); which can lead to localized depletion, especially for high value species such as *P. imperator*. Little is known about the reproduction of many of the populations of larger angelfish species including *P. imperator*, with the few studies that have been conducted focusing mainly on systematics, courtship and spawning, with some attention on reproduction in other Pomacanthid species (Konow *et al*., 2006; Aburto-Oropeza *et al*., 2000; Thresher, 1982).

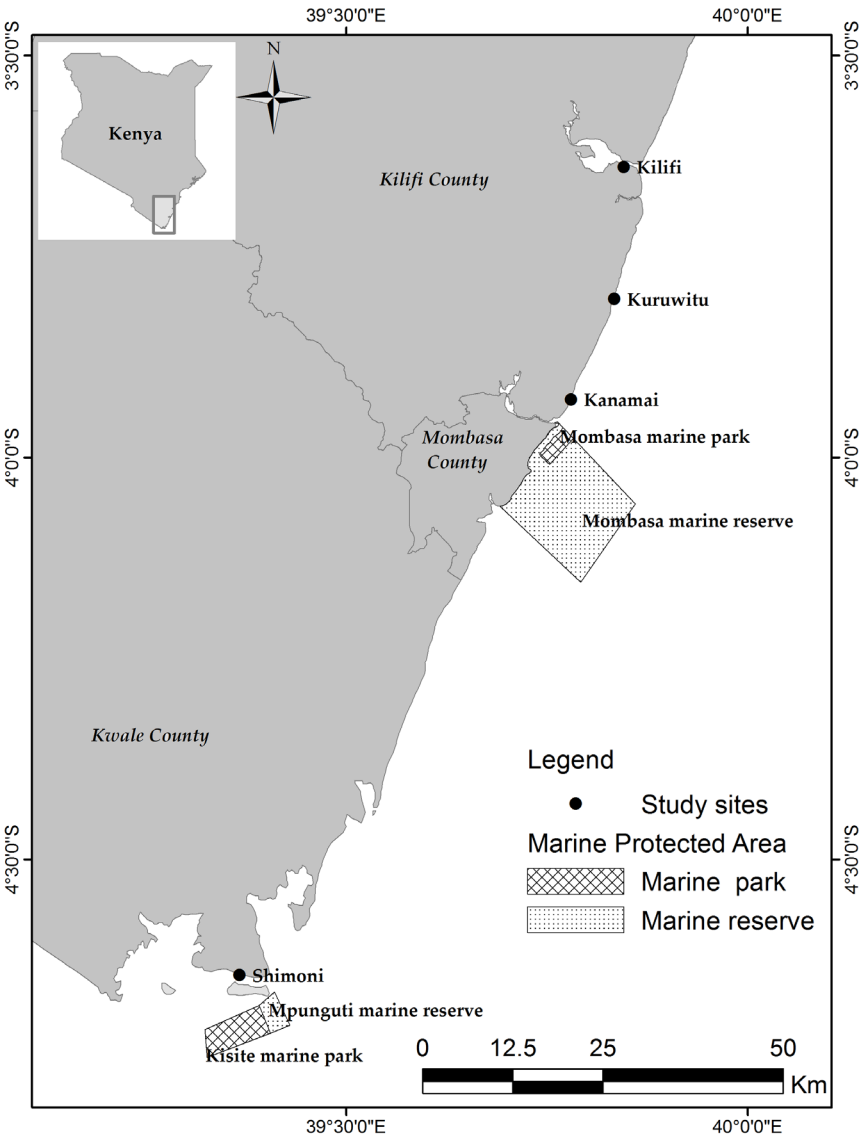

Figure 1. A map of Kenya (inset) showing the location of study sites of Shimoni, Kanamai, Kuruwitu and Kilifi.

Trade in marine angelfishes is almost entirely comprised of wild-caught specimens (Collier *et al*., 2003). *P. imperator* is highly sort after and among the most valued (Okemwa *et al*., 2009). Chung and Woo (1999) affirm that commercial exploitation of *P. imperator* has only focused on the aquarium trade and therefore individuals longer than 25cm (SL) are rarely exploited. *P. imperator* is among the species that has been reported to have declined due to the aquarium trade globally (Schwerdtner and Dandava, 2014), and in Kenya (Okemwa *et al*., 2006). A significant catch is taken along the Kenya coast by artisanal fishers even though the species is predominantly targeted for the aquarium trade, and may therefore be under heavy fishing pressure. Artisanal fishers targeting this species mostly use spear guns and hand lines (Obota, pers. obs., 2014). Selective harvesting of specific sizes may affect reproductive capacity and population structure and thus reduce resilience to growth and recruitment overfishing (Jennings *et al*., 2001). *P. imperator* was ranked as moderately vulnerable to over-exploitation by the aquarium fishery in a risk assessment study using productivity and susceptibility analysis (PSA) (Okemwa *et al*., 2016). The species has the potential for localized depletion under the cumulative pressures of both the aquarium and small-scale food fish fisheries. Due to the lack of adequate information on stocks of *P. imperator*, the design of sound management interventions and harvest strategies has been difficult. This study presents the first investigation into the reproductive biology and body condition of *P. imperator* populations along the Kenyan coast, as a first step towards evaluating their stock status. The aim of this study was to

provide baseline biological information as a contribution towards enhancing the management of key target species exploited by the aquarium fishery in Kenya.

Materials and Methods

Sampling sites

The sampling sites for this study were located in Shimoni on the south coast of Kenya (04° 38.9´ S; 39° 22.9´ E), and Kilifi on the north coast (3° 38.3´ S; 39° 50.77´ E). Specimens were collected at Kilifi, Kuruwitu and Kanamai landing sites in Kilifi County, and at Shimoni village in Kwale County (Fig. 1). The sites were purposely selected because they are among the most important fishing grounds for aquarium fish species in terms of volume (Okemwa *et al.,* 2016).

Fish Sample collection and specimen processing

Due to variability in the artisanal catch, samples were only collected for the months of March through July 2014, and January through June 2015. Total length (TL)

Table 1. Criteria used for determination of maturity stages of *P. imperator* (adapted from Murphy and Taylor [1999], West [1990], and Yamaguchi *et. al.* [2006]).

(a)

Ovarian maturity stage	Macroscopic features
Immature	Ovary small, strand-like and transparent; Most advanced oocytes (eggs) are at peri-nucleus stage or yolk vesicle stage.
Maturing	The gonads present reduced dimension (6mm in length and 1.8cm width). Ovaries in color from pink to pale yellow. Most oocytes are in early vitellogenesis stage. Oocytes in late vitellogenesis stage can also be seen. Besides, brown bodies can also be observed.
Ripening	Ovaries are yellow in color. An increase in blood vessels, volume and size structure are recorded. Most oocytes are in the late vitellogenesis stage. Few are in early vitellogenesis stage. Brown bodies are also registered.
Spawning	Gonad very developed. Ovary coloration varies from yellow to orange. Migrating nucleus, hydrating or hydrated oocytes visible through wall; typical of individuals just prior to spawning; egg release possible with application of light abdominal pressure. Brown bodies recorded.
Post-spawn / Resting	Most advanced oocytes (eggs) are at peri-nucleus stage or yolk vesicle stage. Due to the diameter of the gonad and the thickness of the gonad wall, it is possible to differ the resting ovaries from the immature ones.

(b)

Maturity stage of testis	
Immature/ inactive	Difficult to determine sex macroscopically. Testis small, threadlike and transparent. Testis with spermatogonia in the first spermatogenesis stage.
Maturing	Testis transparent or pale white. Tissue predominantly comprised of primary and secondary spermatocytes. Few quantities of Spermatids in lobules.
Ripening	White testis. Tissue consists predominantly of spermatocytes, Spermatids and spermatozoa. Spermatozoa in lobules but none in spermatic ducts.
Spawning	White testis enlarged. Mature spermatozoa fill the spermatic ducts.
Post- spawn / Spent	Testis dull brown in color. Developed lobules containing few remaining sperms. Flat, white-grayish testes spermagonia in first spermatogenesis

was measured to 1mm precision while body weight (BW) was measured to 0.01g precision. The specimens were transported to the laboratory and frozen for further analysis.

Laboratory Work

All gonads were removed by dissecting through the body cavity as shown in Plate 3, then weighed on a digital top-loading balance (TX223L, SHIMADZU, Japan) to 0.001g precision. The gonads were then preserved in Boin's solution before final analysis (Saborido-Rey & Murua, 2003). The histological procedures used in this study followed Wu *et al*. (2008). After fixation, each pair of ovaries or testes was drained of excess fixative through sequential immersion in different concentrations of ethanol (70%, 80%, 95%, and 100%), then cleared in xylol, and embedded in paraffin. The ovaries were then sectioned at 5µm using a rotary microtome. The sections were mounted on slides and stained with haematoxylin and counter stained with eosin. The sectioned and stained tissues were subsequently examined under a light microscope for gonadal maturation staging. The ovaries were then preserved in modified Gilson's fluid (Simpson, 1959) for the measurement of ova diameter and fecundity studies.

Sex ratio

A non-parametric Chi-square (χ^2) test was used to examine the homogeneity of sex ratio. Data was tabulated by month and by length class distribution to assess monthly and size related trends in the sex ratio of the population. The significance of the deviation of the sex ratio from the expected 1:1 ratio was determined using the non-parametric chi-square test (Zar, 2010).

Examination of gonads

Macroscopic examination of gonads was conducted to determine the sex of each individual and weight to 0.001gm accuracy. The gonad maturity stages were categorized following the method adapted from Murphy and Taylor (1999), West (1990), and Yamaguchi *et al.* (2006). Five maturity stages; Immature (I), Maturing (II), Mature (III), Ripe (IV), Spawning (V) and Spent (VI) were recorded based on macroscopic examination of gonads (Table 1). Maturity stages were discerned from colour and size of the oocytes. The number of male and female *P. imperator* sampled was recorded for each survey.

Determination of spawning season

To determine the spawning season for *P. imperator*, BW and gonad weight (GW) were taken for all specimens sampled during the study period. Percentage of GW in relation to the BW was calculated since the development of gonads and general fish growth are associated, as the gonado-somatic index (GSI), using the following formula: GSI = [GW / Gutted BW] x 100 (Barber and Blake, 2006).

Fecundity

Portions of the lobes in stage III to VI (Maturing, Spawning and Spent) were cut from the middle of the ovaries and weighed. The portions were then stored in Gilson's fluid for determination of fecundity and ova diameter (Saborido-Rey and Murua, 2003). After about 48 hours in preservative, the eggs were completely

Table 2. Monthly sex ratio for emperor angelfish, *P. imperator*, from March through July 2014, and January through June 2015.

	Male	Female	Sex ratio	P-*value*
14 Mar	8	10	1:1.25	0.6374
14 Apr	3	5	1:1.67	0.4795
14 May	7	4	1:0.57	0.3657
14 Jun	1	3	1:3	------
14 Jul	2	6	1:3	0.1573
15 Jan	9	6	1:0.67	0.4386
15 Feb	13	9	1:1.69	0.3938
15 Mar	10	27	1:2.7	0.0052
15 Apr	23	61	1:2.6	0.0001
15 May	20	22	1:1.1	0.7576
15 Jun	38	23	1:0.61	0.0548
Overall	134	176	1:1.3	0.0171

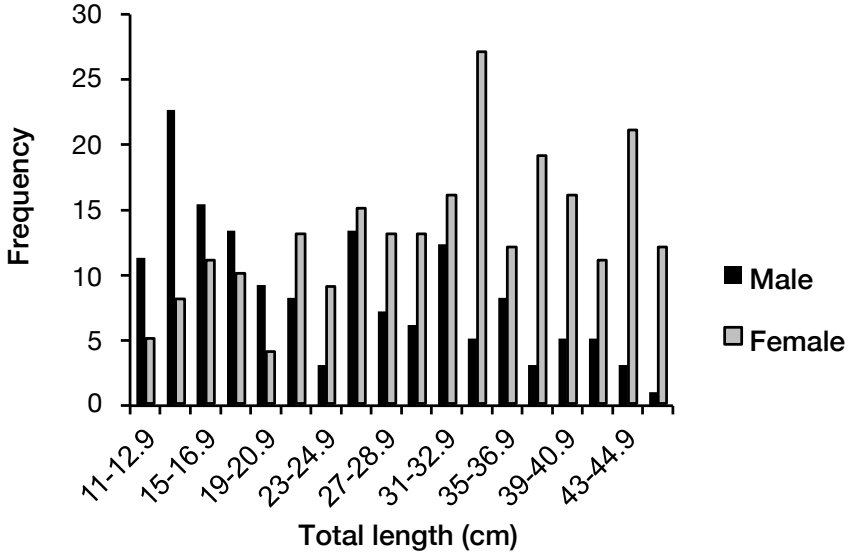

Figure 2. Length-frequency distribution of male and female *P. imperator* collected along the Kenyan coast.

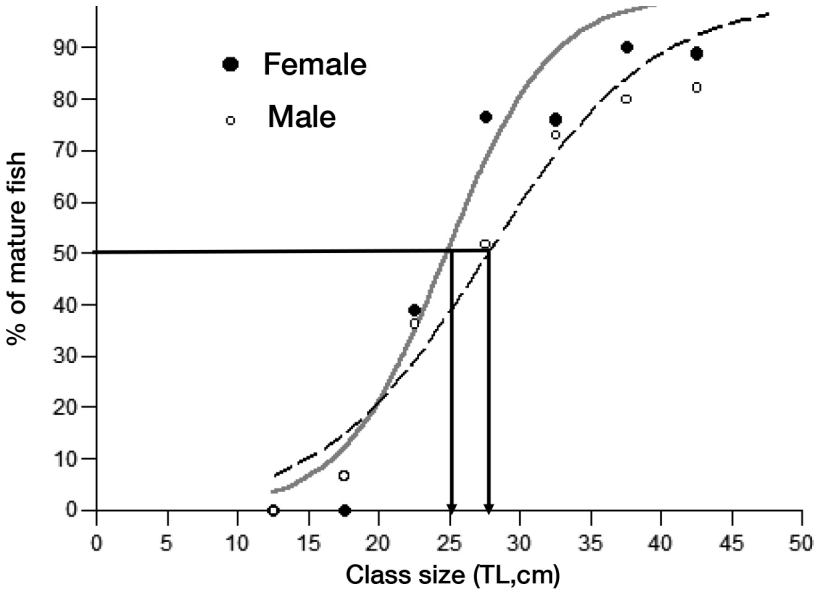

Figure 3. Cumulative percentage of observed mature fish in relation to body size for females (continuous line) and males (dashed-line) of *P. imperator.* The lines show the estimated size where 50% of fish were mature.

released from the tissues after vigorous shaking. For determination of ova diameter, portions of the ovary taken from different regions of the ovary were placed on a glass slide, teased out and later examined under a light microscope. Ova diameter was measured using an ocular micrometer with 0.029mm divisions where each ovum was taken in the same parallel plane using the mechanical stage of the microscope to avoid errors due to distortion and subjective bias due to irregular shape of the ova. All the oocytes in the sub-sample of the ovary were counted and measured using a calibrated eye-piece graticule under a microscope at a magnification of ×40. Fecundity was calculated as follows: BF= nV/v × W/w where n is the number of oocytes in the sub-sample, v is the volume of the sub-sample, V is the volume of sample, W is the weight of the ovary, and w is the weight of the sub-sample. The least squares method was then used to determine the relationship between fecundity, total length, and gonad weight.

Size at maturity

The L_{50} in this study was defined as the length at which 50% of individuals in a given length-class reached maturity. Size at sexual maturity was

Table 3. Determined estimates of batch fecundity, ovary weight, total length, and body weight of *P. imperator*.

	Fecundity	Ovary weight (g)	Body weight (g)	Total length (cm)
Minimum	17,790	10.9	770	26.6
Maximum	266,472	47.4	2114	40.5
Mean	79353±11,747	25.2±2.1	1202.6± 66.3	33.28± 0.7

modeled using a logistic equation by including the asymptotic limit found in the current fish size data. The following equation was curve-fitted using Delta Graph Win (Version 5.6.2) to obtain the L_{50}: M (TL) = 100/(1+exp (-a*(x-b))). L_{50} was estimated by initially calculating the coefficients *a* and *b* respectively, by maximizing the likelihood of binomial distribution where *a* is a constant and *b* is the L_{50}. Specimens with ovaries belonging to maturity stage III -VI (Ripening, Spawning and Spent) were considered as mature.

Length-Weight Relationship (LWR) and condition factor (*Kn*)

The LWR was estimated by the equation: $W = aL^b$ where 'W' is body weight (BW), 'L' is total length (TL), 'a' and 'b' are constants. The exponent (b) of the monthly length-weight relationship for *P. imperator* was tested for significant deviation from the isometric value of *b*=3 following (Froese and Pauly, 2006). Relative condition (*Kn*) factor was calculated following Le Cren (1951) where: $K_n = W/\hat{W}$. W is the observed weight and $\hat{W}$ is the weight calculated from the length-weight relationship as $\hat{W}=aL^{3-b}$. Data was pooled based on similarity in slope of the regression of the length-weight relationship between the sexes as detected by ANCOVA.

Monthly K_n values were calculated for each sex and plotted to test for seasonal changes as defined by Froese and Pauly (2006). All statistical tests were considered at a significant level of 95% (α=0.05). Monthly LWR values were calculated for each sex and plotted as an indicator for seasonal changes as defined by Froese and Pauly (2006).

Results

Sex ratio and size composition

A total of 384 specimens (126 males, 192 females, and 66 unsexed) were sampled from the small-scale fisheries in this study. The overall sex ratio of *P. imperator* (1:1.3) differed significantly from the expected ratio of 1:1 (χ^2 = 19.07, p<0.05) with a predominance of females in the sampled population (Table 2). The distribution of monthly frequency of occurrence of males and females shows no significant difference in sex ratio except in March and April 2015. On average, females were larger than males and the majority of the specimens larger than 21 cm were females, as shown in length frequency distribution in Fig. 2.

Size at maturity (L_{50})

The total length at sexual maturity, $L_{50,}$ was estimated as 25cm for females and 28cm for males (Fig. 3). The females of *P. imperator* therefore attained gonadal

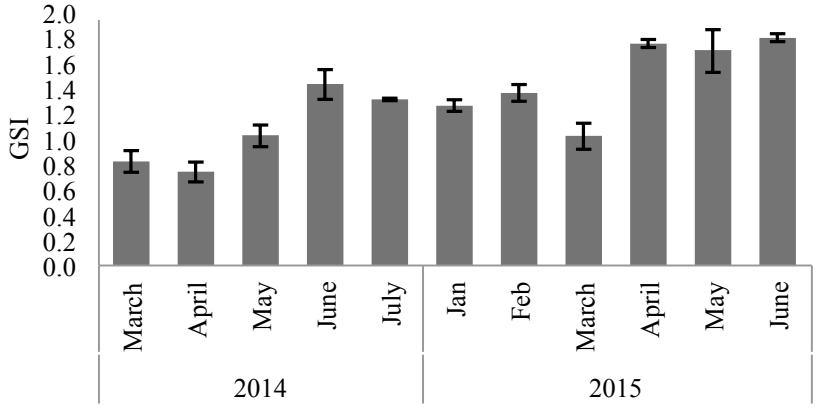

Figure 4. Monthly trends in Gonado-somatic Index (GSI) of female *P. imperator* along the Kenyan coast.

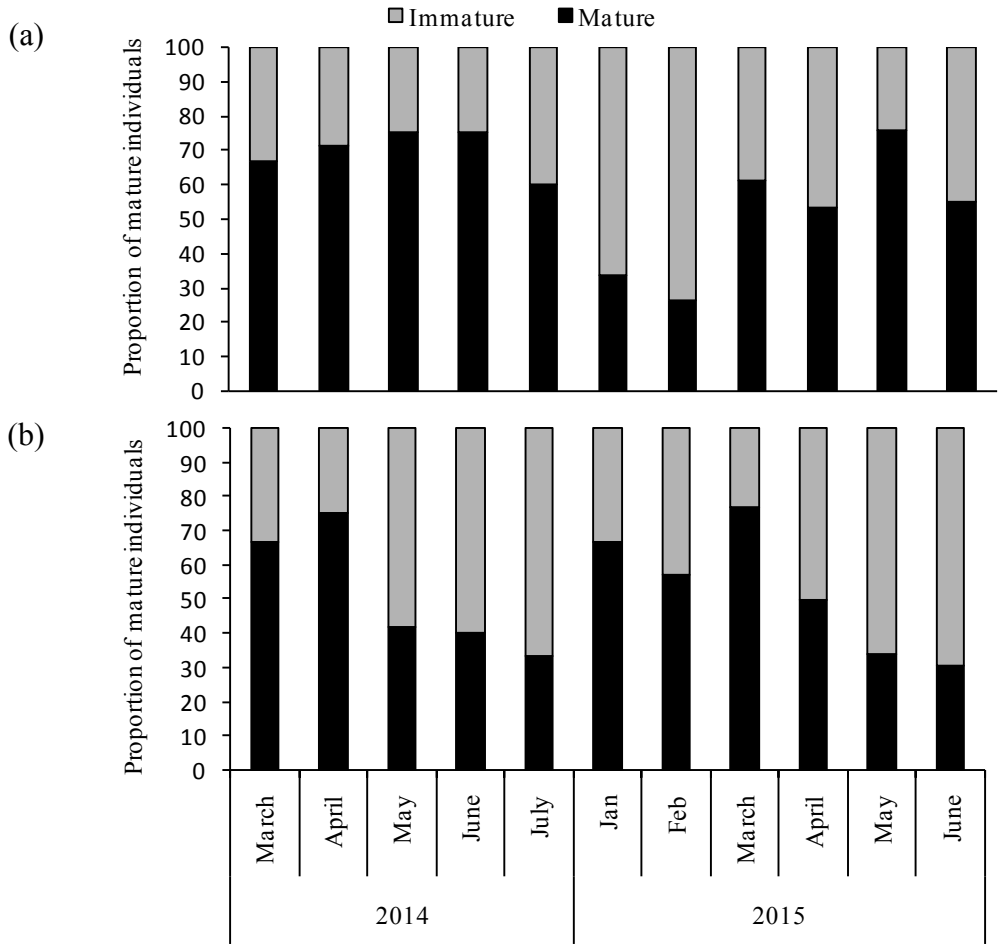

Figure 5. Distribution of mature individuals of *P. imperator* during the sampling period. Mature (Stage III –IV) and Immature (Stage I – II). Female (a) and male (b).

maturity at a smaller body lengths than males. Specimens were identified as mature if gonads were found to be Ripe, Spawning, or Resting.

Gonad development

The mean monthly values of GSI of maturing and mature females are plotted in Figure 4. The GSI of mature females was highest during the month of April 2016. June 2015 and May and June 2016 also recorded high GSI values. Frequency of monthly gonadal maturation stages of females indicates that mature females occurred throughout the sampled months as shown in Fig. 5. *P. imperator* has a prolonged reproductive period, almost throughout the sampled months, with a peak from March to April 2016.

Ovary diameter and fecundity

Polymodal frequency diameter was found in the ovaries in the mature stages (III-VI) as shown in Fig. 6. During stages III and IV, the smallest oocyte diameter ranged between 0.3-0.7 mm. Size ranges of mature oocytes in stage V and VI was 0.3 - 0.8 mm. The estimated fecundity, ovary weight, total length, and body weight are given in Table 3. The estimated fecundity ranged from 17,790 in females of 26.6cm to 266,472 eggs in females measuring 40.5cm, with a mean of 79,353±11,747 (Mean ±SE). Total length is closely correlated with fecundity as shown in Fig. 7 (r^2=0.89). Total length of *P. imperator* can therefore be used as a good estimator of fecundity. The Fecundity-Total Length and Fecundity-Ovary weight relationship was represented by the following equations in this study:

Fecundity-Total Length: F= 0.000001TL7.1855, r^2 = 0.888
Fecundity-Ovary weight: F=1678.3TL1.1581, r^2 = 0.537

Length weight relationship and condition factor

The length weight relationship for *P. imperator* males and females was expressed as:
Males: Log W= - (1.553) + 3.083 log L, or W= 0.022*$L^{3.083}$
Females: Log W= - (1.157) + 2.772 log, or W= 0.003 * $L^{2.772}$
The *b* value was 3.083 for males and 2.772 for females.

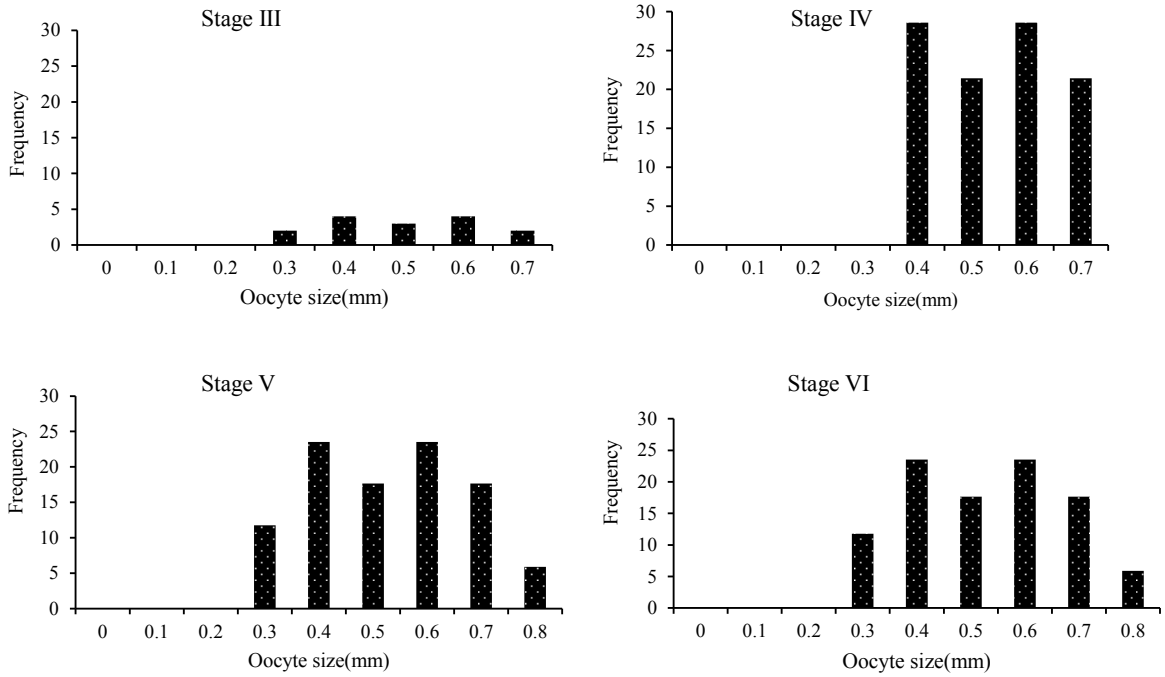

Figure 6. Oocyte size-frequency distributions of *P. imperator* from samples collected along the Kenyan coast.

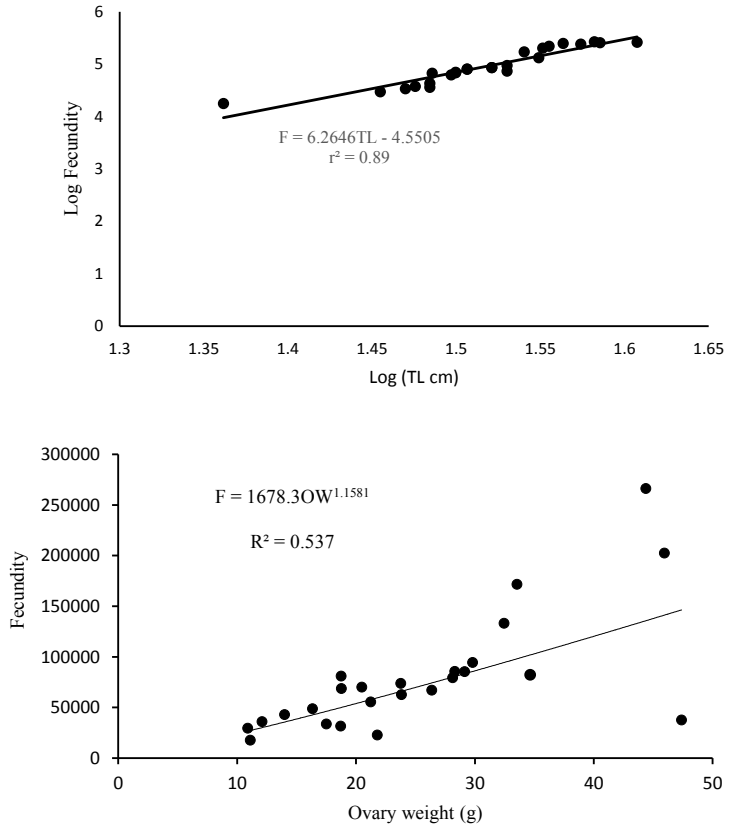

Figure 7. Variations in fecundity with total length (above) and ovary weight (below) for the *P. imperator* along the Kenyan coast.

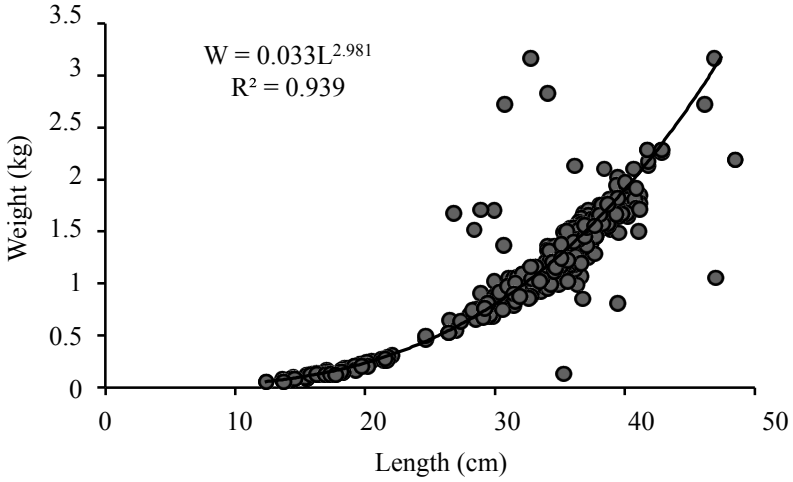

Figure 8. Length-weight relationship of *P. imperator* for pooled samples of both sexes during the study period.

Correlation coefficient, r, between the logs transformed for length and weight data, was found to be 0.825 in males and 0.878 in females. Significant differences in both slope (*b)* and intercept (*a*) were not observed between sexes (ANCOVA, *p=0.18*). Therefore, the length-weight relationship for the pooled data was best described by the equation: W= 0.033 * $L^{2.981}$ (n=384) as shown in Fig. 8. LWR indicated isometric growth both in males and females, as the allometric coefficient *b* values were not significantly different from the expected isometric value of 3 (Student's t-test, *p=0.12*).

Condition factor

The Relative condition factors (K_n) for both sexes did not vary monthly. The monthly (K_n) ranged from 1.8 to 4.6 in males and from 2.8 to 4.4 in females. Throughout the sampled months the relative condition factor was above 1 for both male and female *P. imperator*. The highest values for both males and females were recorded in July.

Discussion

In the present study, females of *P. imperator* were generally larger in size than males, although their size distribution overlapped. Chung and Woo (1999) obtained similar results where males dominated the smaller sizes and females were generally larger. This size distribution pattern may be an indication of an aggregation for females at the sampled sites. The sex-ratio distribution was consistent with other studies on the genus *Pomacanthus* (Michael, 2004). Studies by Arellano-Martinez *et al*. (1999) noted that in *Holocanthus passer*, the sex ratio differed significantly with females dominating the smaller, and males the larger size classes. Feitosa (2009) (b also found males to be larger than females in *P. paru* and he suggests that when females attain sexual maturity, they reduce their growth rate. Allen *et al.* (1998) provided the explanation that *P. paru* occurs in the Atlantic and *P. imperator* occurs in the Central and Indo-Pacific, and therefore environmental and genetic characteristics could also explain the difference in growth parameters. Nonetheless, the observed sexual dimorphism in this species is typical for Pomacanthidae. The dominance of females in the larger size classes observed may be due to aggregation for spawning, or differential growth rates. It is known that late maturing individuals in a population of fish start channeling more energy towards reproduction than early maturing ones, which may contribute to the predominance of females in the larger size classes. The present study suggests that males mature at a larger size than females.

This study has suggested the size at maturity (L_{50}) for male and female *P. imperator* is 25 cm and 28 cm respectively. The males of *P. imperator* matured earlier than the females, probably because they require less energy for gonad maturation. Feitosa (2009) reported that size at maturity for female *P. paru* was 30cm, and 35cm for males, which is inconsistent with this study. This can be attributed to environmental and genetic factors that influence size at maturity of fish, but fishing pressure may also affect this parameter (Jennings *et al.,* 2001).

The diameter of oocytes ranged between 0.3mm and 0.8mm in all the mature stages of *P. imperator* (stage III-VI). It is therefore possible that the ova present in

the ovary develop and are released in batches. The fecundity estimate of *P. imperator* in the present study ranged between 17,790 - 266,472, slightly lower than a species of the same genus (*P. paru*) in north-eastern Brazil which was reported by Feitosa (2009) to reproduce approximately 126,000 eggs during the peak spawning months of May, July, August, October and November. The average fecundity obtained in this study is much higher than that of *P. paru* described by Aiken (1983) who found a mean value of 34,200. Similarities are recorded between the present study and studies by Arellano-Martinez *et al*. (2006) for *P. zonipectus* who found a mean value of 79,400±9,200. Fecundity is a specific reproductive trait and is adapted to the life cycle of the species, varying with size, growth, population density, body food availability, and mortality rate; the life-history traits that represent trade-offs in evolutionary terms.

Gonado-somatic index is an indicator of spawning season but caution should be taken when assessing for spatial and temporal variations due to regional and temporal physiological differences (Jons and Miranda, 1997). Seasonal variation in GSI was analyzed alongside other factors such as monthly proportion of fish in various stages of gonadal development (Garcia-Cagide *et al*., 2001). The occurrence of Mature (III), Ripe (IV), Spawning (V) and Spent (VI) maturity stages observed throughout the study period, and in greater proportions during March and April, is an indication that *P. imperator* is a continuous spawner with peak spawning in March and April. This conforms to the findings of (Munro *et al.,* 1973) with regards to other Indian Ocean teleost. Continuous spawning was noted in other *Pomacanthus* as reported by (Feitosa, 2009) who indicated that *P. paru* could be spawning all year round, with evidence of ripe individuals throughout the year, but with peaks in May, July, August, October and November. The same study also indicated that the peak spawning for *Holocanthus ciliaris* extended from the month of January through August. Munro *et al.* (1973) also recorded a maximum proportion of *P. aucuatus* with mature gonads in October and January. The peak spawning season for *P. imperator* (March-April) seemed to fall within the North East Monsoon period (November to April) when the East African coastal waters are calm due to the absence of the fast moving East African Equatorial Current that is common during the South East Monsoon period (June-July). A favorable environment for the survival of eggs, larval and post larval stages seem to be the main drivers in spawning seasonality in this species. Mature individuals were observed in all months during the study period. This may suggest that *P. imperator* spawns continuously with a peak in March/April.

The value of *b* (3.038) found in this study was close to the theoretical value *(b* = 3) indicating isometric growth, occurring at the same rate for both length and weight. The parameters of the length-weight relationship are influenced by a series of factors including season, habitat, gonad maturity, sex, diet, and stomach fullness. The allometric coefficient (*b*) generally lies between 2.5 and 3.5 (Froese and Pauly, 2006) in fishes. The values for both male and female *P. imperator* during this study were within this range. Differences in *b* values can be attributed to a combination of one or more factors including habitat, area, season, stomach fullness, gonadal condition, sex, health, preservation methods, and differences in the size of specimens (Hossain *et al.,* 2011). Not all of these factors were accounted for in this study. It appears that K_n of *P. imperator* did not vary significantly throughout the study period ($p>0.05$).

Conclusions

This study established that *P. imperator* can be categorized as sexually dimorphic, gonochoristic, and that the size at sexual maturity of female and male *P. imperator* is 25cm and 28cm respectively. This study has estimated the fecundity of *P. imperator* to range from 17,790 - 266,472 eggs. The polymodal oocyte diameter distribution indicates that oocyte development in the ovary of *P. imperator* is asynchronous. While the GSI may not be very accurate in estimating spawning period, together with the proportion of mature individuals in all the sampled months, *P. imperator* has been found to have a spawning period throughout the sample period with peaks in March and April. The relationship between total length and body weight suggests that *P. imperator* exhibits isometric growth. There is a need for further research to obtain more robust data throughout the full calendar year, to determine whether spawning in *P. imperator* may have two annual peaks.

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