

**REPRODUCTIVE BIOLOGY OF EXPLOITED POPULATIONS OF THE  
EMPEROR ANGELFISH, *Pomacanthus imperator* BLOCH, 1787 ALONG THE  
KENYAN COAST**

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A thesis submitted in partial fulfillment of the requirements for the Degree of Master of  
Science in Fisheries of Pwani University

**MAY, 2016**

**DECLARATION**

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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**DEDICATION**

This thesis is dedicated to my K'Ochero family, classmates and friends who stood by me.

I will always remember your words of encouragement and support when I needed you.

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## ABSTRACT

Substantial proportion of the *Pomacanthus imperator* are traded in terms of value and quantity and also harvested as food in the artisanal fishery in Kenya. However information on their reproductive biology is scanty. In this study, we investigated the reproductive biology of the *P. imperator* by collecting specimen from small-scale fishers in selected landing sites along the Kenyan coast for a period of 11 months from March 2014 to July 2014 and from January 2015 to June 2015. A total of 384 specimens (126 males, 192 females and 66 unsexed) were analyzed. Overall sex ratios were significantly differently from 1:1 according to chi-square test ( $p < 0.05$ ). Sizes at maturity ( $L_{50}$ ) were estimated to be 25cm and 28cm in TL for females and males respectively. Fecundity was estimated to be in the range of 17,790-266,472 with a Mean  $\pm$ SE of  $79,353 \pm 11,747$  and, was linearly related to total length and ovary weight. *P. imperator* showed extended spawning period which peaked gradually between March-April based on estimations of the gonad somatic index and monthly proportion of mature individuals. The length-weight relationships (LWRs) for males was best expressed by  $\text{Log } W = -1.553 + 3.022 \log \text{ TL}$  and for females:  $\text{Log } W = -1.157 + 2.772 \log \text{ TL}$ . However, significant difference in both slope ( $b$ ) and intercept ( $a$ ) was not observed between sexes (ANCOVA;  $p = 0.18$ ) and therefore, subsequent analysis for Length-weight were pooled. LWR indicated isometric growth both in males and females *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 found from this study provide crucial baseline information for management of this species which has shown high vulnerability to depletion due to overfishing.

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## CHAPTER 1: INTRODUCTION

### 1.1 Background information

Angelfishes belong to the family Pomacanthidae. Angelfishes of the genus *Pomacanthus* are among the most highly prized ornamental reef fishes traded worldwide, and are a favorite fishes for divers and aquarists (Thresher, 1982; Debelius *et al.*, 2003; Nelson, 2006). The family Pomacanthidae accounts for about 25% by volume of the estimated 1,472 aquarium species traded annually (Sadovy, 1996; Wabnitz *et al.*, 2003). In Kenya, the genus forms a substantial proportion of the aquarium fish trade in terms of value and quantity traded.

The Emperor angelfish, *Pomacanthus imperator* is among the most spectacularly colored and broadly recognized species of coral reef-associated fishes. It is found on tropical reef bottoms and is widely distributed in the Indo-pacific region (Murugan and Durgekar, 2008). Chung and Woo (1999) affirm that the commercial exploitation of *P. imperator* has only focused on the aquarium trade and therefore individuals longer than 25cm (SL) are rarely exploited.

In Kenya, the coastal and marine fisheries can be divided into three main categories: (i) the small-scale fisheries employing majority of the fishers who use traditional fishing methods, often on foot or with non-mechanized vessels driven by paddles and sails, (ii) the sport or recreational fisheries associated with the tourism industry, and (iii) the aquarium fisheries targeting the aquarium fish for both local and export markets among other fisheries such as trawlers and long line fisheries. According to Okemwa *et al.* (2009), the aquarium fisheries and fish-trade in Kenya can be traced to the 1960s. Since then the sector has expanded tremendously, with the number of traded species from about

48 species in the 1980s (Samoilys, 1988) to over 200 species currently (Okemwa *et al.*, 2009). The main export markets include USA, UK and Europe. Although the fishery is conducted throughout the year, relatively high landings are recorded during the months of September through March, coinciding with North East Monsoon (NEM) season (GoK, 2009; Okemwa *et al.*, 2009). Knowledge of the status of majority of the aquarium fish populations is however lacking due to limited data and information on the biology and life history of target species. As a result, designing species-based sustainable exploitation strategies has been difficult.

The exploitation patterns in the ornamental fisheries in Kenya focus on specific fishing grounds and selected species (Okemwa *et al.*, 2016) which can lead to localized depletion especially for high value species such as *P. imperator*. Extended research efforts should therefore focus on establishing the key biological parameters including size at maturity, spawning season, fecundity and sex-ratio in order to aid in formulation of sound management measures for the ornamental fisheries in Kenya.

## **1.2 Problem Statement and rationale**

Angelfishes are among the ten fish families that constitute 94% of the aquarium fish traded in Kenya (Okemwa *et al.*, 2016). Among the angelfishes, *P. imperator* is one of the most valued and targeted species (Okemwa *et al.*, 2011). The smaller individuals are exploited for aquarium purposes while the adults are captured as food-fish by artisanal fishers, thereby subjecting the species to high exploitation pressure. Artisanal fishers targeting this species mostly use spearguns and handlines. Spearguns fishers are selective in terms of the sizes they catch, mostly targeting the larger individuals, while handlines catch broad length-size ranges, including spawning and breeding individuals. Selective

harvesting of specific sizes may therefore impact the reproductive capacity and population structure and, thus reduce resilience from growth and recruitment overfishing (Wotton, 1998; Jennings *et al.*, 2001). Due to the lack of adequate information, the design of sound management interventions is also hampered. In assessing the status of *P. imperator* stocks, there is a need for information on the reproductive biology.

A recent risk assessment using productivity and susceptibility analysis (PSA) by Okemwa *et al.* (2016) ranked *P. imperator* as moderately vulnerable to over-exploitation in the aquarium fishery. Therefore the potential for localized depletion for this species would be higher due to the cumulative effect from both the aquarium and small-scale food fish fisheries along the coast which target this species.

Knowledge on the reproductive biology and life history of exploited fish populations is vital especially for the management of fisheries, with parameters such as size at maturity and fecundity being of critical importance (Wotton, 1998; Jennings *et al.*, 2001). This study presents the first investigation into the reproductive biology of the *P. imperator* populations along the Kenya coast, and is geared as a first step towards the evaluation of the stock status of this important species.

### **1.3 Aim**

The aim of this study was to provide baseline biological information as a contribution towards enhancing the management of key species exploited by the aquarium fishery in Kenya

#### **1.4 Objectives**

The broad objective of this study was to describe the reproductive biology of exploited populations of *P. imperator* along the Kenyan coast.

The specific objectives of the study were:

1. To determine the monthly variation in the sex ratio of *P. imperator* along the Kenyan Coast.
2. To establish the size at maturity, fecundity and spawning seasons of *P. imperator* along the Kenyan Coast.
3. To determine the length-weight relationship and body condition for *P. imperator* in the studied populations along the Kenyan Coast.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 General Biology and distribution of Angelfishes

The emperor angelfish *P. imperator* belongs to the group of marine coral reef fishes in the family Pomacanthidae and order Perciformes (Nelson, 2006). There are 88 recognized species in this family, belonging to eight genera worldwide: *Pomacanthus*, *Holocanthus*, *Centropyge*, *Chaetodontoplus*, *Euxiphipops*, *Genicanthus*, *Paracentropy* and *Pygoplites* (Debelius *et al.*, 2003). According to Eschmeyer *et al.*, 2016, the phylogeny of *P. imperator* is summarized in Table 1.

**Table 1:** Phylogeny of *P. imperator*

Kingdom	Animalia
Phylum	Chordata
Order	Perciformes
Class	Actinopterygii
Family	Pomacanthidae
Genus	<i>Pomacanthus</i>
Species	<i>P. imperator</i>

The family Pomacanthidae generally exhibits a circum-tropical distribution, with majority of species inhabiting shallow reefs of less than 30m depths, characterized by coral, sponge or rocky substrates (Thresher, 1984; Debelius *et al.*, 2003). Geographically, *Pomacanthus* species are widely distributed in the western Indo-Pacific waters (Fricke, 1999; Pyle, 2001). In particular, *P. imperator* has a wide Indo-Pacific distribution from the Red sea and East Africa to the Hawaiian, Line and Tuamoto Islands. The species can also be found in southern Japan and south to the Great Barrier Reef and a single record from Hawaii (Froese and Pauly, 2006; Randall, 2007). The source of this specimen could be a possible escapee from an aquarium, since *P. imperator* is a popular species in the aquarium trade (Golani *et al.*, 2010). The *P. imperator* inhabits outer coral reef or rocky habitat at depths

of 5-60 m. They live solitary or in pairs, the male defending aggressively its territory against co-specific males (Heemstra and Heemstram, 2004).

The color of the juvenile and the adult *P. imperator* are very different as evident in Plate 1 and 2. The juveniles are dark blue overall with concentric white circles on the body and lines on the head while adults are yellow with blue lines on the body and a black eye bar. Juveniles begin to take on some of the markings of the adult as they grow, which gradually replace all the juvenile color characteristics.



Plate 1: Juvenile *Pomacanthus imperator*



Plate 2: Adult *P. imperator*

Fricke (1980) speculates that the distinctive coloration of *P. imperator* provides a kind of 'intra-specific camouflage' that allows the juveniles to go unrecognized as competitors by the adults. Thresher (1984) further suggested that distinct coloration in juvenile fishes may reduce attacks by territorial adult's conspecifics, thereby enabling juveniles to access resources found within territories.

Most studies on the reproductive biology of *Pomacanthus* angelfishes are on the smaller "pygmy" angelfishes of the genus *Centropyge*, and mostly, in captivity (Sakai *et al.*, 2003). Close similarities have been documented on Pomacanthid reproductive biology and in phylogenetic relationships and have led some authors to assume protogynous



hermaphroditism to be universal in the family (Neudecker and Lobel 1982; Moyer, 1987). However, no direct proof of hermaphroditism has been obtained in the genus *Pomacanthus*. The reproductive biology of other species of angelfishes has been well studied in both the Eastern and Central Pacific Ocean regions. However, literature on the same species in the Western Indian Ocean (WIO) region is clearly lacking (Arellano-Martinez *et al.*, 1999). In the studied angelfish species, spawning occurs during the warmer months in the temperate regions (Thresher, 1984). For instance, in the Gulf of California, breeding in *P. zonipectus* is reported to occur from July to September (Thomson, 1987). Similarly, the reproductive cycle of *P. scalare* shows a clear seasonality related to changes in water temperature indicating a single breeding season between March and July (Farida *et al.*, 2013). Very little has been reported on the reproduction of many of the larger angelfish species including *P. imperator*. The few studies conducted on *P. imperator* mainly focused on courtship and spawning, with some comments on the reproduction in other Pomacanthid fishes (Thresher, 1982). In a review of the biology of the larger angelfish species, many of the species in the genus *Pomacanthus* showed similarities in reproductive attributes such as paired spawning, courtship spawning rituals and pelagic release of eggs (Thresher, 1984). Studies on the genera *Holocanthus* and *Pomacanthus* suggest that, courtship and spawning occur at dusk in all species and is preceded by male display to the female, although this has not clearly been associated with lunar periodicity since the diel timing of reproduction has differed within the *Pomacanthus* species (Thresher, 1982; Moyer *et al.*, 1983).

Arellano *et al.* (1999) suggested that there is a habitat preference between sexes in *P. Paru* females preferring sheltered areas for spawning. Feitosa (2009) described the population

biology of *P. Paru* and indicated that *P. Paru* is a gonochoristic fish exhibiting size-related differences in sexes. The study showed that *P. Paru* has an extended spawning season with peaks in March-April and September-October. Sex ratio of angelfish populations seems to be determined by the mating system, protogynous hermaphroditism, fish migration for food and aggregation during spawning. Aburto- Oropeza *et al.* (2000) noted significant difference between the sexes in *P. Paru* over the size range, with females prevailing at smaller sizes and males at larger sizes (Thresher, 1984; Arellano-Martinez *et al.*, 1999).

Some attempts have been made in assessing the biology of *Pomacanthus* species in other waters. The most noteworthy study is on age and growth of *P. imperator* which documented a maximum standard length (SL) of 41.19 cm (Chung and Woo, 1999). In a separate study in the Red sea, Thresher (1982) found that *P. imperator* exhibited size differences in relation to sexes. Thresher (1984) also recorded differences in size and colour between the sexes of *Pomacanthus* angelfishes. There is little documentation of seasonal spawning and sequential hermaphroditism in *P. imperator*. Moyer *et al.* (1983) also noted that males grow to larger sizes compared to females. The variations in sex-ratios is thought to be as a result of seasonal factors related to climate, environmental variability in the fishing grounds, differential mortalities, difference in growth, and longevity among sexes (Micale *et al.*, 2002, Chiou *et al.*, 2004, Koutrakis *et al.*, 2004). In addition, sexual dimorphism, and migration patterns may also influence sex-ratio in fish population (WU *et al.*, 2008).

## **2.2 Spawning season and gonadal development**

Reproductive activity in angelfishes is strongly seasonal (Thresher, 1984). Among the key environmental factors that regulate reproductive activity in reef fishes is water temperature which acts as a spawning trigger and influences the rate of gonadal development (Moyle and Cech, 1996; Bye, 1990) observed that a sudden increase in water temperature appeared to trigger maturation and ovulation in most species of angelfish. On the other hand, an increase in water temperature has been documented to slow down maturation and evolution in other species such as *Mugil cephalus*, *Decentrarchus labrax*, and *Sparus auratus* (Zohar, 1989). Furthermore, inter-annual variations are also common as reported in other coral reef fishes (Farida *et al.*, 2013). Most angelfish species exhibit extended spawning seasons, although the intensity of spawning activity, as determined by direct observation of spawning, also varies seasonally (Thresher, 1984).

## **2.3 Size at maturity**

Some species of angelfish are protogynous hermaphrodites changing from females to males at later age (Thresher, 1984, Sakai *et al.*, 2003). This may explain the larger sizes in males compared to females at the first maturity of the species (Moyer and Nakazono 1978; Randall and Yasuda 1979). Similar observations, of larger males among species exhibiting hermaphroditism in many reef fishes have been reported in several studies (Warner, 1988; Sadovy, 1996; Jennings *et al.*, 2001).

## **2.4 Fecundity**

Fecundity in reef fishes varies by species, age, size, and seasonal environmental conditions, which influence food availability and thus the individual growth rate (Farida *et al.*, 2013). Different reproductive strategies are reflected by marked differences in

fecundity among species (Murua and Saborido-Rey, 2003). Fecundity may also vary within a given species as a result of different adaptations to environmental habitats (Witthames *et al.*, 1995). Fecundity is documented to vary annually even within the same stock, and is proportional to fish body condition and size with larger fish producing more eggs (both in relative and absolute terms) per body mass (Kjesbu *et al.*, 1991). At the population level, fecundity may also decline as a consequence of reduced abundance of spawners and disproportionate reduction in the large highly fecund females, especially in heavily exploited populations. In such scenarios, large old fish are rapidly eliminated because they are more exposed to size-selective fishing mortality (Trippel *et al.*, 1997).

### **2.5 Length-Weight Relationship and Condition Factor**

Knowledge of length-weight relationship (LWR) and Relative condition factor ( $K_n$ ) of fishes is important in fisheries science. LWR has important applications in fish stock assessment provide valuable information on the habitat where the fish lives (Morey *et al.*, 2003; Dan-Kishiya, 2013). Therefore, data on a well-designed LWR of fish species is important for fish stock assessment and parameters  $a$  and  $b$  can be used for length-weight conversion. At the same time, the relationship of length and weight estimates condition factor ( $K_n$ ) of the fish species and fish biomass through length frequency (Dan-Kishiya, 2013). Therefore, for a given size, females in better condition have higher fecundity, thus fish condition can be a key parameter to assess fecundity at the population level. The condition factor in fish serves as an indicator of physiological state of the fish in relation to its welfare (Le Cren, 1951; Dan-Kishiya, 2013). Index of relative condition factor can also be useful in monitoring feeding intensity, and age and growth rates (Ndimele *et al.*, 2010). Imam *et al.* (2010) suggests that condition factor is important in understanding the

life-cycle of fish species and therefore, it contributes towards management of the species. These variations in the life-history characteristics of the species, between population of the same species, between seasons and different years suggest that the effects of fishing also vary between species. Consequently, some species may be more resilient to fishing pressure than others, thereby calling for specific management strategies that do not assume uniform life history characteristics among species even within the same genus.

## **CHAPTER 3: MATERIALS AND METHODS**

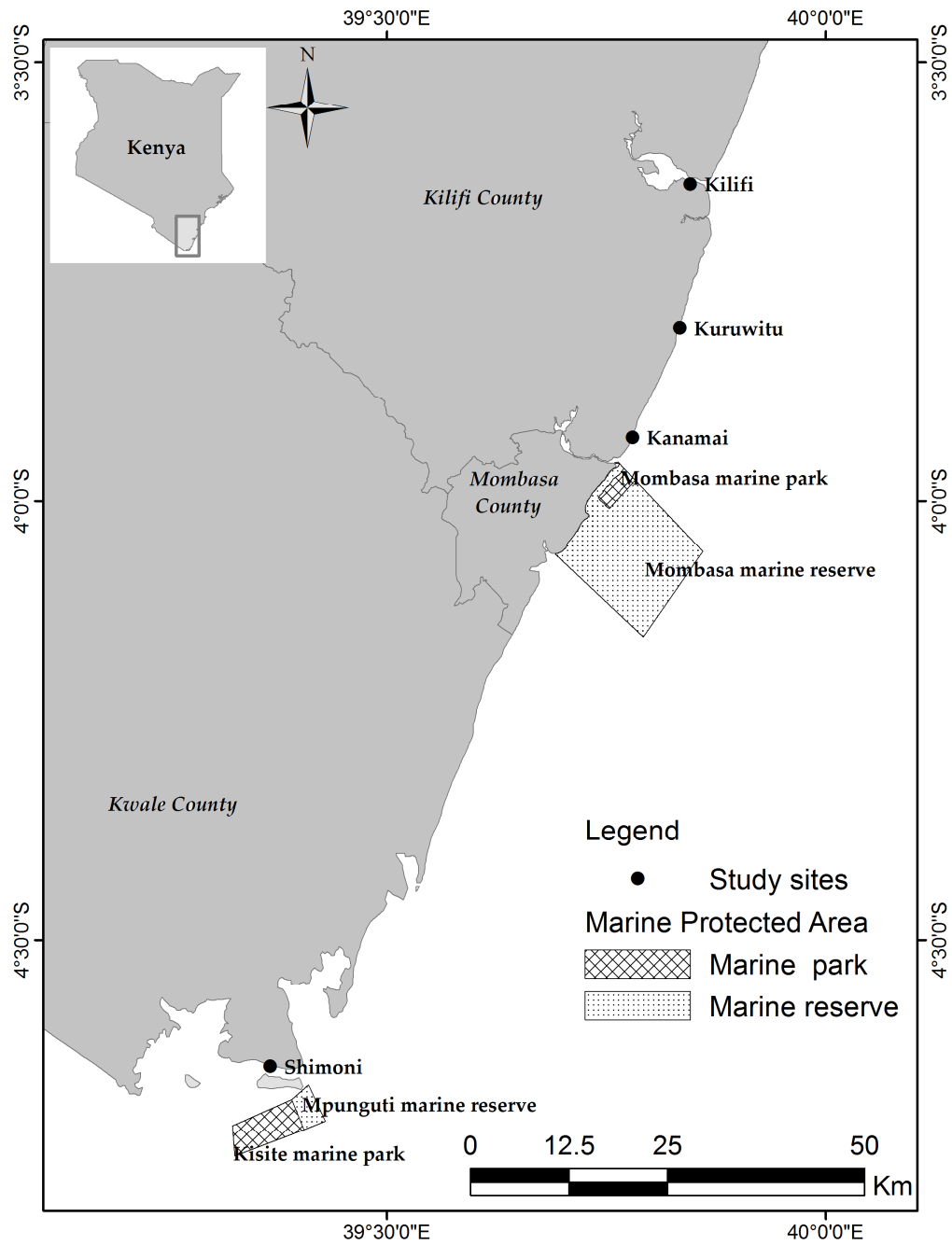
### **3.1 Description of the sampling sites**

The sampling sites for this study included Shimoni in the south Kenya Coast straddling 04° 38.9' S and 39° 22.9' E, and Kilifi in the north, at 3° 38.3' S and 39° 50.77' E where most of the specimen were collected. Sampling sites included Kilifi, Kuruwitu and Kanamai Landing sites in Kilifi County in the North of Kenyan Coast where more samples were collected and Shimoni in Kwale County (Figure 1). The sites were purposively selected because they are among the most important fishing grounds for aquarium fish species in terms of volume (Okemwa *et al.*, 2016). Shimoni area encompasses the Pemba channel, a stretch of rich fishing grounds separating the Eastern Africa coast and Pemba Island. Part of the Shimoni traditional fishing grounds have been gazetted as a marine protected area (MPA) under the Kisite-Mpunguti Marine National Park and Reserve (KMMNP and R).

### **3.2 Fish Sample collection and specimen processing**

The samples were collected on a monthly basis from March to July 2014 and January to June 2015 and data recorded in a form (Appendix 1). Once fish was landed, *P. imperator* was identified and sorted from the catch. Total length (TL) of *P. imperator* was measured to 1mm precision using a 1m wooden measuring board. Body weight (BW) was measured using a portable electronic weighing scale (WeiHeng 40/10, Japan) to 0.01g precision. The

specimens were transported to the laboratory and frozen for further analysis.



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

### 3.2.1 Laboratory Work

All gonads were removed by dissecting through the body cavity as shown in Plate 3 then weighed on a digital top-loading weighing balance (TX223L, SHIMADZU, Japan) to 0.001g precision. The gonads were then preserved in Boin's solution before final analysis. The histological procedures used in this study followed Wu *et al.* (2008). After fixation, each pair of ovaries or testes was drained of the excess fixative in different levels of ethanol sequentially (70%, 80%, 95%, and 100%), then cleared in xylol and embedded in paraffin. The ovaries were then sectioned at 5 $\mu$ m 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.

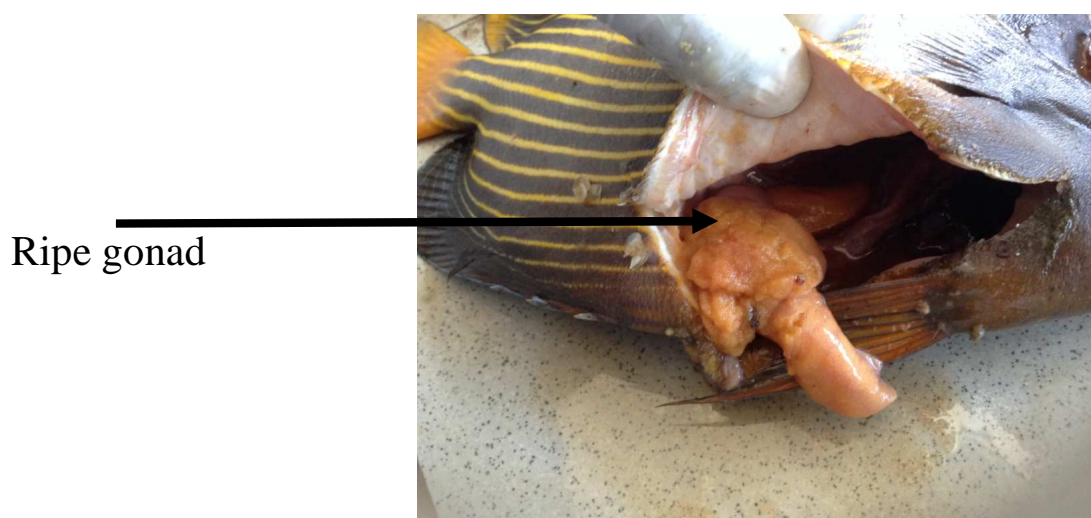


**Plate 3:** Dissection of a specimen in the laboratory



### 3.3 Sex ratio and Sex determination

Sex was determined in 318 out of 384 fish samples collected during the study period by visually examining the gonads. The gonads of *P. imperator* are located just above the alimentary canal in the abdominal cavity and both the testis and ovary are two-lobed (Plate 4). The two lobes form a U-shape and are open to the outside by a common duct.



**Plate 4:** Location of the abdominal incision on a *P. imperator*, used to visually examine the gonads during sex identification

A non-parametric Chi-square ( $\chi^2$ ) test was used to examine the homogeneity of the sex ratio. Data was tabulated by month and by length class to assess monthly and size related trends in the ratio of the population. The significance of the deviation of the sex ratio from the expected 1:1 ratio was determined using non parametric chi-square test (Zar, 2000).

### 3.4 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 2). 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.

**Table 2:** Criteria used for determination of maturity stages of *P. imperator* [(a) Ovary and (b) Testis. Adapted from Murphy and Taylor (1999); West (1990) and Yamaguchi *et al.* (2006)

(a)	
Ovarian maturity stage	Macroscopic features
Stage I: Immature	Ovary small, strand-like and transparent; Most advanced oocytes (eggs) are at peri-nucleus stage or yolk vesicle stage.
Stage II: 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.
Stage III: 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.
Stage IV-V: 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.
Stage VI: 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	

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

### 3.5 Determination of spawning season

To determine the spawning season for *P. imperator*, body weight (BW) and gonad weight (GW) were taken for all specimens sampled during the study period each month. Percentage of GW in relation to the BW was calculated since the development of gonads and the general fish growth are associated, as the Gonad-somatic index (GSI), using the following formula:-

$$\text{GSI} = [\text{GW} / \text{Gutted BW}] \times 100 \text{ (Barber and Blake 2006) ----- (Eq. 1)}$$

where GW = gonad weight and BW = body weight

### 3.6 Fecundity

One lobe of the ovary lobes was stored in Bouin's fixative for histological examination and portions of the other 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).

Ovary samples fixed in the Gilson's fluid was teased out and dispersed in small amounts of water, then shaken periodically while in the fluid to help loosen the ovarian tissue and to ensure rapid penetration of the preservative. After about 48 hours in preservative, it was expected that the eggs were completely released from the tissues during the vigorous shaking. For determination of ova diameter, portions of the ovary taken from different regions of the ovary were placed on a glass slide and teased out then 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. To estimate fecundity, the Gilson's fluid was replaced with water and the eggs washed repeatedly while decanting the supernatant. All the oocytes in the sub-sample of the ovary piece were counted measured using a calibrated eye-piece graticule under a microscope at a magnification of  $\times 40$ . Fecundity was calculated as follows:

$$BF = \frac{nV}{v} \times \frac{W}{w} \text{ ----- (Eq. 2)}$$

where n is the number of oocytes in the sub-sample, v is the volume of sub-sample, V is the volume of sample, and 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, and total length, and gonad weight.

### 3.7 Size at maturity

The logistic equation was used to estimate Length at massive maturity ( $L_{50}$ ). The  $L_{50}$  in this study was defined as length at which 50% of individuals in a given length-class reach maturity. Size at sexual maturity was 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  $L_{50}$ .

$$M(TL) = 100 / (1 + \exp(-a \cdot (x - b))) \text{ ----- (Eq. 3)}$$

$L_{50}$  was estimated by initially calculating the coefficients  $a$  and  $b$  respectively, in the logistic equation 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.

### 3.8 Length-Weight Relationship (LWR) and condition factor ( $Kn$ )

Length and weight was measured for a total of 384 specimens for determination of length-weight relationship (LWR) and condition factor. The LWR was estimated by the equation

$$W = aL^b \text{ (Allen 1985)}$$

where 'W' is body weight (BW), 'L' is total length (TL), 'a' and 'b' are constants.

The growth models used in fish population dynamics assume isometric growth (i.e. exponent of the length-weight relationship,  $b=3$ ). Therefore, 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):

$$K_n = W/\hat{W} \text{ ----- (Eq. 4)}$$

W is the observed weight and  $\hat{W}$  is the weight calculated from the length-weight relationship as  $\hat{W}=aL^{3-b}$ .

Monthly  $K_n$  values were calculated for each sex and plotted as an indicator for seasonal changes as defined by (Froese and Pauly, 2006). All statistical tests were considered at a significant level of 95% ( $\alpha=0.05$ ) for all statistical tests in this study.

## CHAPTER 4: RESULTS

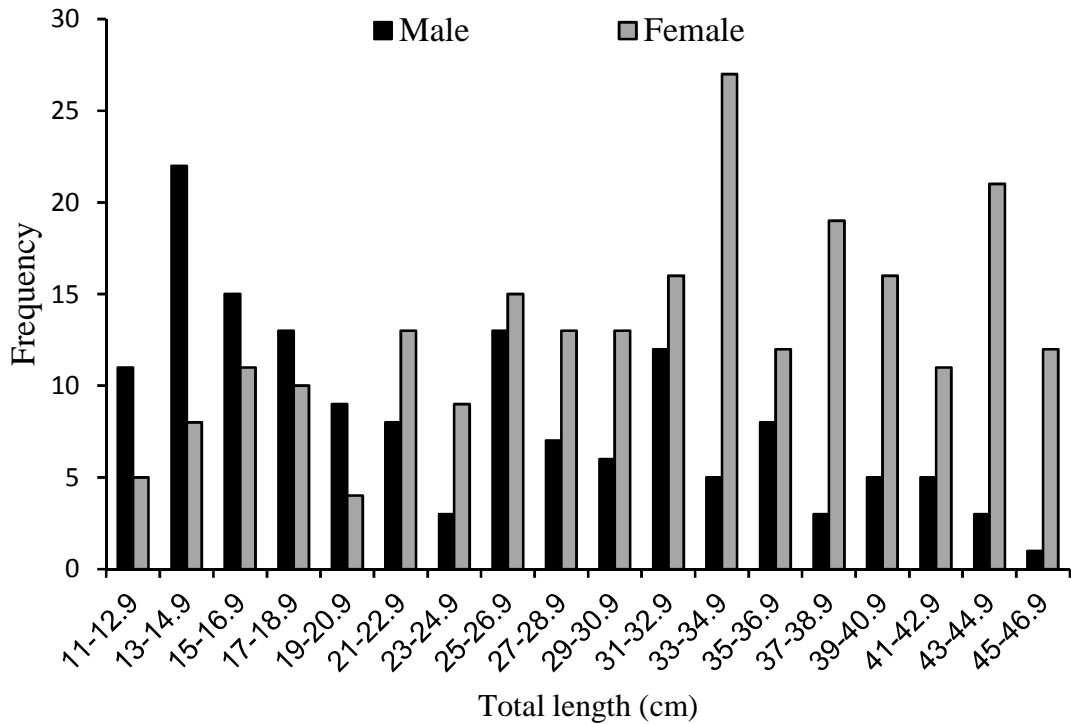
### 4.1 Sex ratio and size composition

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 ( $\chi^2 = 19.07$ ,  $p < 0.05$ ) with 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 of *P. imperator* except in March and April 2015. Chi square test was not calculated for June 2014 since the number of individuals collected did not meet the minimum expected count of 5 necessary for  $\chi^2$  test.

**Table 3:** Monthly sex ratio for *P. imperator* from March through July 2014 and January through June 2015

	Male	Female	Sex ratio	<i>P</i> -value
Mar-14	8	10	1:1.25	0.6374
Apr-14	3	5	1:1.67	0.4795
May-14	7	4	1:0.57	0.3657
Jun-14	1	3	1:3	-----
Jul-14	2	6	1:3	0.1573
Jan-15	9	6	1:0.67	0.4386
Feb-15	13	9	1:0.69	0.3938
Mar-15	10	27	1:2.7	0.0052
Apr-15	23	61	1:2.7	0.0001
May-15	20	22	1:1.1	0.7576
Jun-15	38	23	1:0.6	0.0548
Overall	134	176	1:1.3	0.0171

On average, females were larger than males (Paired t-test;  $p < 0.05$ ) and majority of the specimens more than 25 cm were females as shown from the length frequency distribution in Figure 2. There were female-biased size frequencies in the larger size groups especially in size  $\geq 25$ cm.

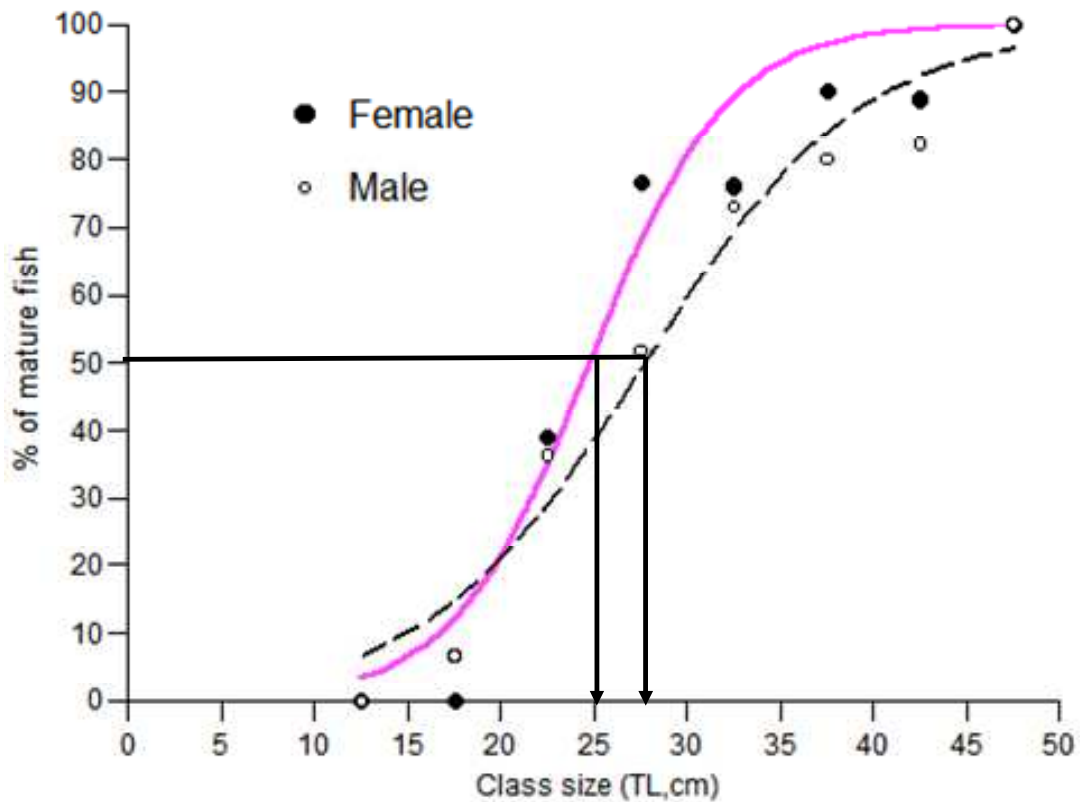


**Figure 2:** Length-frequency distribution of male and female *P. imperator* collected along the Kenyan coast

#### 4.3 Length at massive maturity ( $L_{50}$ )

The total length at sexual maturity,  $L_{50}$  was estimated as 25cm for females and 28cm for males (Figure 3). The females of *P. imperator* therefore attained gonadal maturity at smaller body lengths than males. Specimens were identified as mature (Ripe (IV) + Spawning (V) + Resting (VI)).

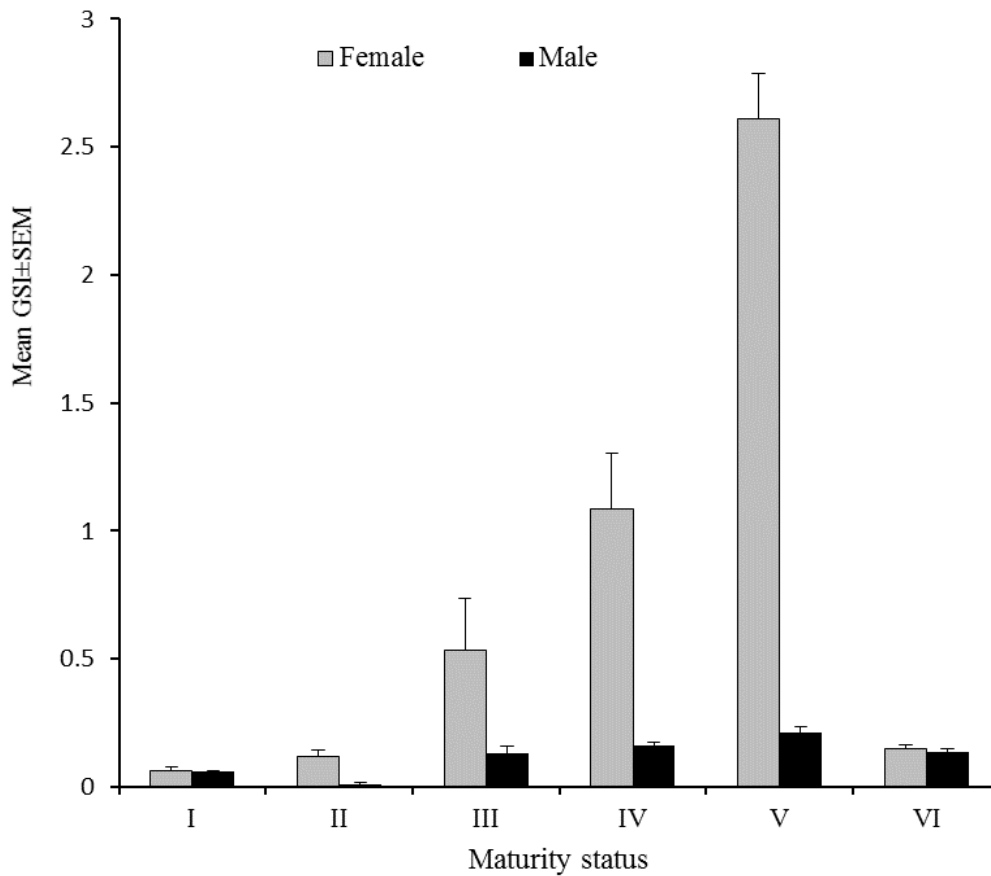




**Figure 3:** Cumulative percentage of observed mature fish in relation to body size for females (continuous line) and males (dashed-line) of *P. imperator*. The lines show the estimated size where 50% of fish were mature

#### 4.4 Gonad Maturity stage

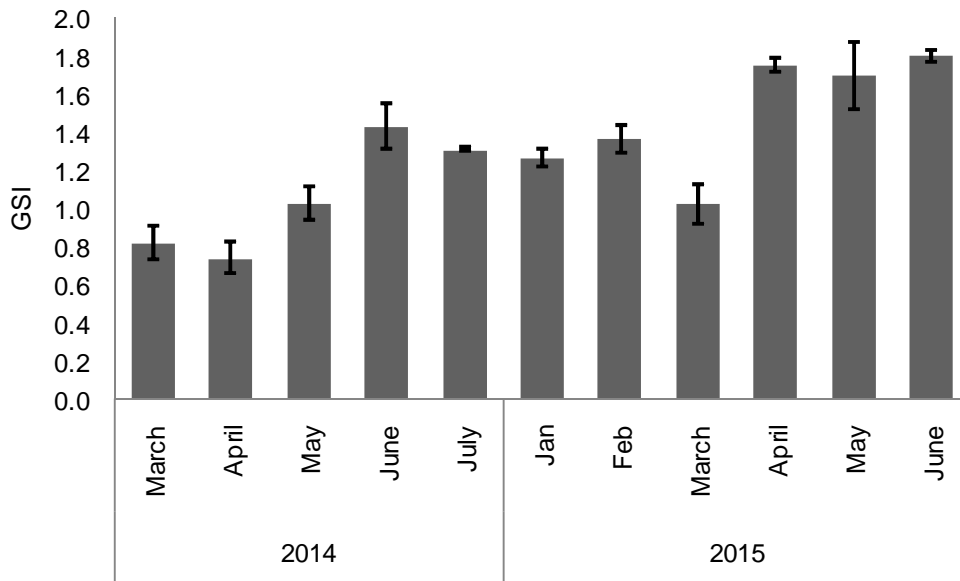
GSI has been taken as additional support for the degrees of confidence for the maturity assessment for both male and female *P. imperator*. The results also show that the GSI was higher for females than males (Figure 4). The GSI increased in both sexes to a peak in stage V followed by a decrease to stage VI. Gradual increase of GSI was recorded during the development of the gonad and declined at the commencement of spawning. Therefore GSI was used to define the spawning seasonality of *P. imperator*.



**Figure 4:** Variation of Gonado-Somatic Index (GSI) with maturity stages in male and female *P. imperator* along the Kenyan Coast

The mean monthly values of GSI of maturing and mature females are plotted in Figure 5.

The GSI of mature females was highest during the month of April 2015. June 2015 and May and June 2014 also recorded high GSI values.



**Figure 5:** Monthly trends in Gonado-somatic Index (GSI) of female *P. imperator*, along the Kenyan Coast

Frequency of monthly gonadal maturation stages of females indicates that mature females occurred throughout the sampled months as shown in Figure 6. *Pomacanthus imperator* has a prolonged reproductive period, almost throughout the sampled months, with a peak from March to April 2015. Mature males of *P. imperator* were also recorded throughout the sampled months with higher proportions of mature individuals in March and April 2014 and March 2015.

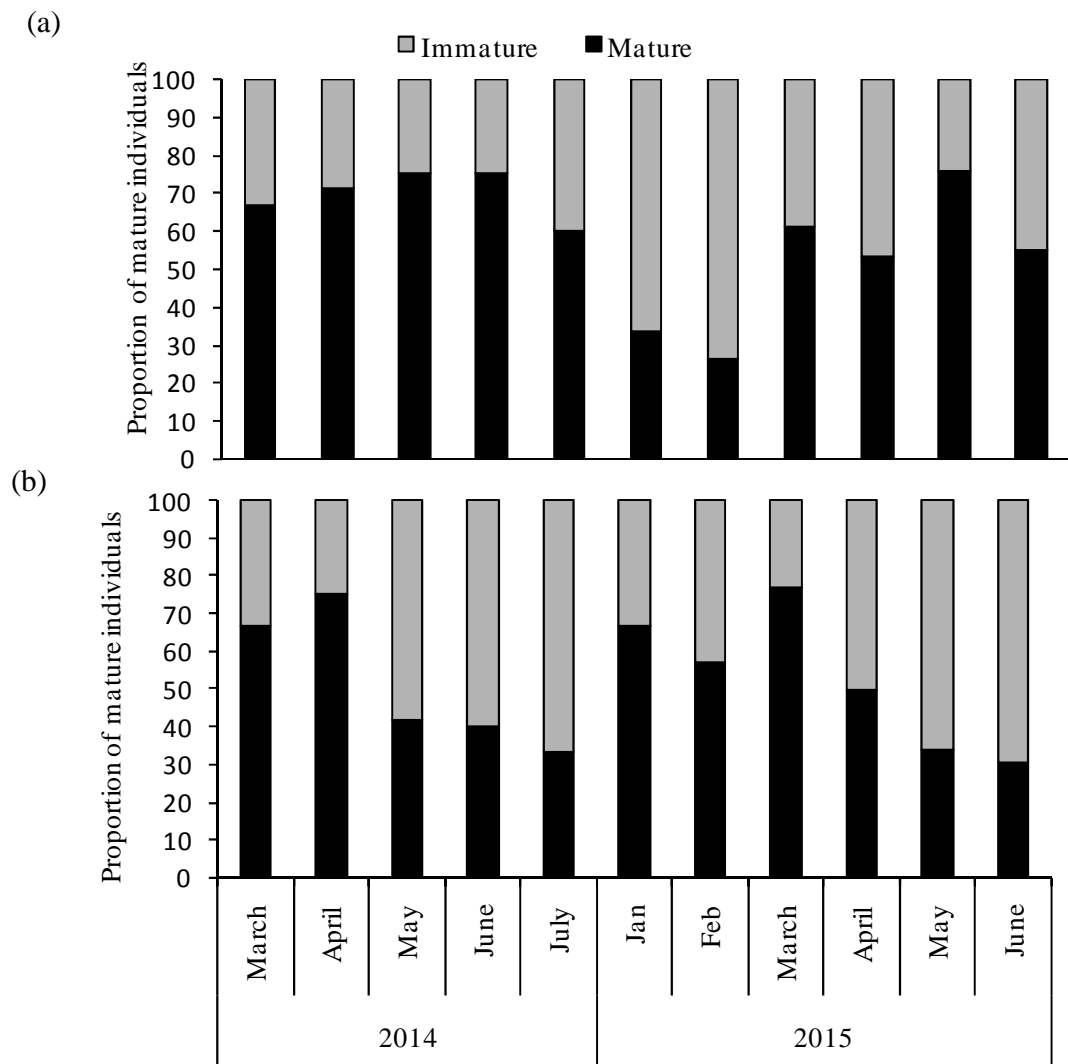
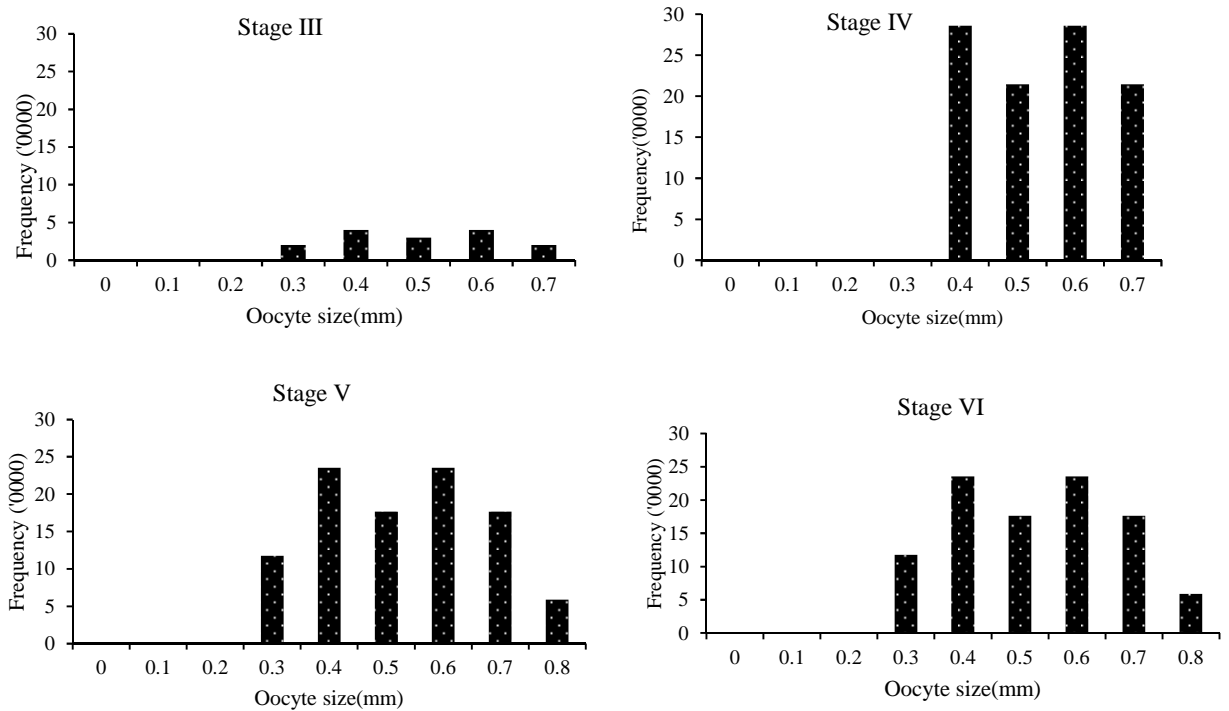


Figure 6: Distribution of mature individuals of *P. imperator* during the sampling period, Mature (Stage III –IV) and Immature (Stage I – II) Female (a) and male (b)

#### 4.5 Ovary diameter and fecundity

Samples of ova taken from the anterior-posterior axis of seven ovaries indicated a relatively uniform size distribution and no significant difference was observed for oocyte sizes in different regions of the ovary (Student's t-test,  $p=0.08$ ). Therefore, ova from mid-portion of the ovary were used in subsequent analysis.

Poly modal frequency diameter was seen in the ovaries in the mature stage (III-VI) as shown in Figure 7. In ovaries with mature 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 oocyte diameters in the mature stages, (III, IV, V and VI) are the same and are therefore released in sequence.



**Figure 7:** Oocyte size-frequency distributions of *P. imperator* from samples collected along the Kenyan Coast

The estimated fecundity, ovary weight, total length, and body weight are given in Table 4. The estimated fecundity ranged from 17,790 in females of 26.6cm to 266,472eggs in females measuring 40.5cm with mean of 79,353±11,747 (Mean ±SE).

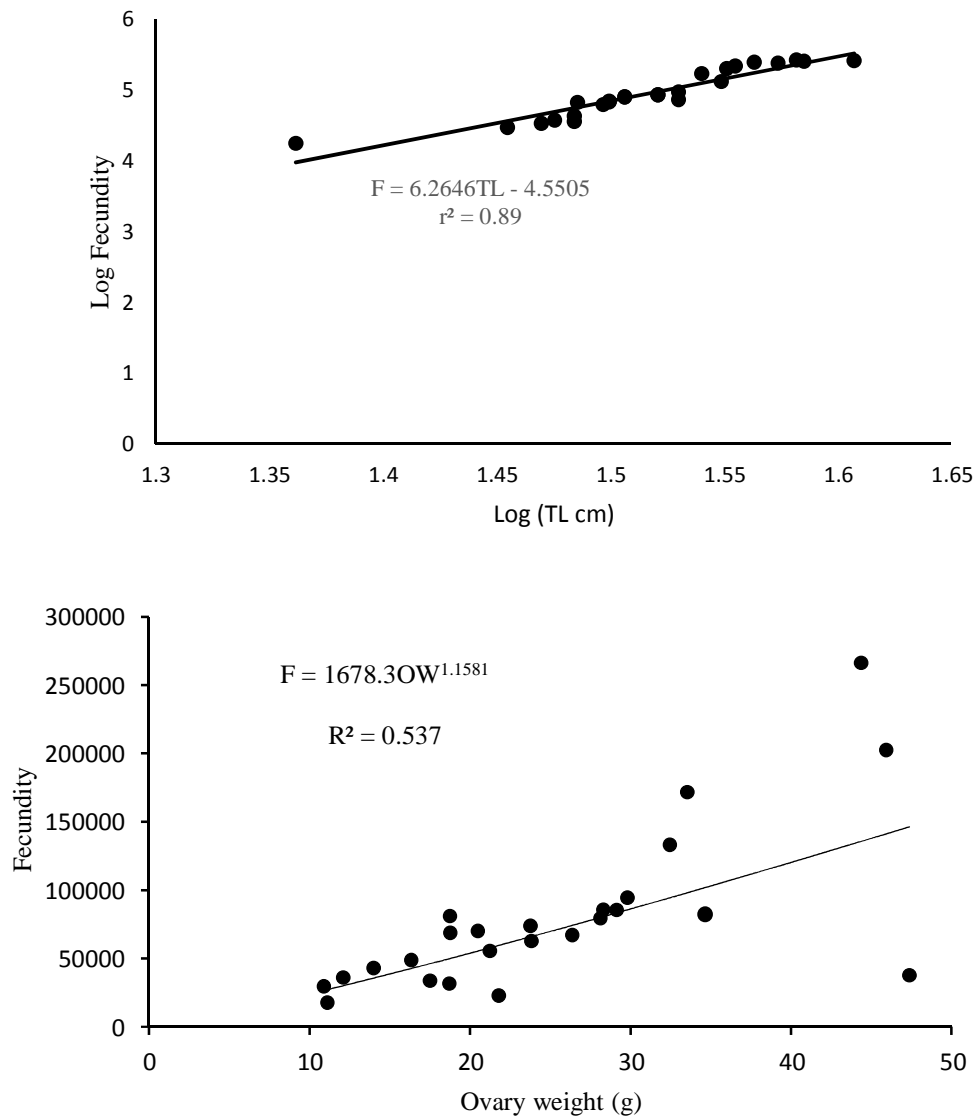
**Table 4:** Determined estimates of batch fecundity, ovary weight, total length, and body weight of *P. imperator*

	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	79,353±11,747	25.2±2.1	1,202.6± 66.3	33.28± 0.7

Total length highly correlated with fecundity as shown in Figure 8 ( $r^2=0.89$ ). 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:

$$\text{Fecundity-Total Length: } F = 6.2646\text{TL} - 4.5505, r^2 = 0.89$$

$$\text{Fecundity-Ovary weight: } F = 1678.3\text{TL}^{1.1581}, r^2 = 0.537$$



**Figure 8:** Variations in fecundity with total length (above) and ovary weight (below) of the *P. imperator* along the Kenyan Coast

#### 4.6 Length weight relationship and condition factor

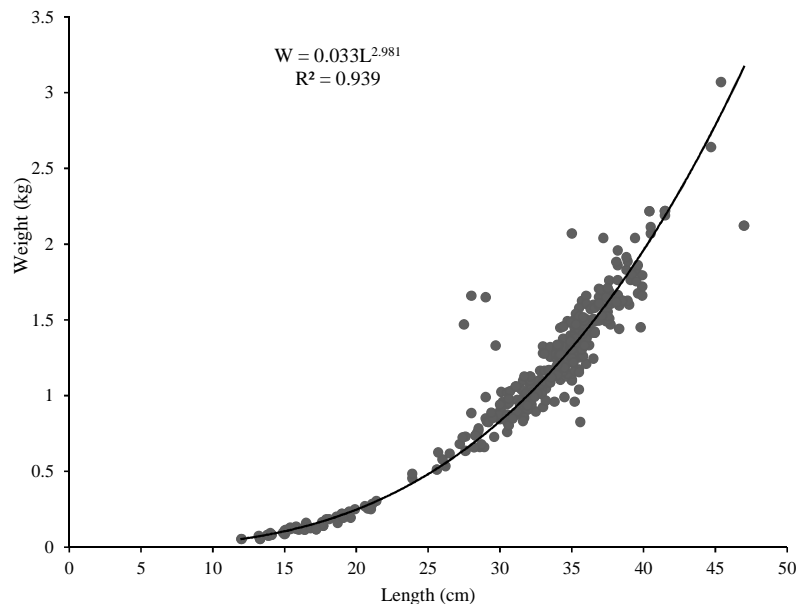
The length weight relationship for *P. imperator* males and females was expressed as:

$$\text{Males: } \text{Log } W = - (1.553) + 3.083 \log L, \text{ or } W = 0.022 * L^{3.083}$$

$$\text{Females: } \text{Log } W = - (1.157) + 2.772 \log L, \text{ or } W = 0.003 * L^{2.772}$$

The  $b$  value was 3.083 for males and 2.772 for females. 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 difference in both slope ( $b$ ) and intercept ( $a$ ) was not observed between sexes (ANCOVA;  $p=0.18$ ). Therefore, length-weight relationship for the pooled data was best described by the equation  $W = 0.033 * L^{2.981}$  ( $n=384$ ) as shown in Figure 9.

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;  $p=0.12$ ).



**Figure 9:** Length-weight relationship of *P. imperator* for pooled samples of both sexes during the study period



The relative condition factors ( $K_n$ ) did not vary monthly for both sexes (Figure 10). 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 male and female were recorded in July. There was no significant variation in the monthly relative condition factor between the sexes (Paired t-test;  $p=0.391$ ).

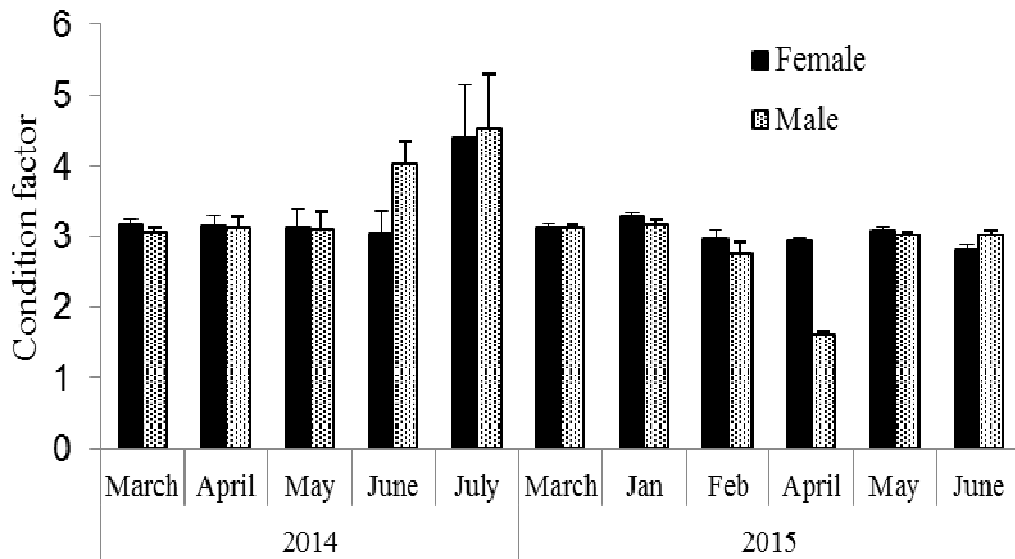


Figure 10: Monthly trends in relative condition factor for male and female *P. imperator*

## CHAPTER 5: DISCUSSION

In the present study, females of *P. imperator* were generally larger in size than males especially during the peak spawning period (February to April) but their size distribution overlapped between the sexes. The sex ratio could be affected by various factors related to fishery, season of the year, shoals in the feeding, and spawning areas. This result of present study supports this pattern based on the size distribution of males and females and the broad overlap. The sex-ratio distribution was consistent with other studies on the genus *Pomacanthus* (Michael, 2004). Chung and Woo (1999) also got similar results where males dominated the smaller sizes and females were generally bigger. This size distribution pattern may be an indication of spawning aggregation for females. Studies by Arellano-Martinez *et al.* (1999) noted that *Holocanthus passer* sex ratio differed significantly with females prevailing the smaller sizes and males the larger lengths. Feitosa (2009) also got similar results where males are larger than females in *P. Paru* and he suggests that when females attain sexual maturity, they reduce their growth. Allen *et al.* (1998) give an explanation that *P. paru* occurs in the Atlantic and *P. imperator* occurs in the Central and Indo-Pacific and therefore environmental and genetic characteristics can 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 stock start channeling more sexual materials much late 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 agrees with the results of studies on *Pomacanthus Paru* (Aiken, 1983; Feitosa, 2009).

This study has established the size at massive maturity ( $L_{50}$ ) for male and female *P. imperator* as 25 cm and 28 cm respectively. The males of *P. imperator* matured earlier than the females, probably because they required lesser quantity of energy reserves for gonad maturation. Feitosa (2009) reported that size at massive 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).

Oocytes diameter ranged between 0.3mm and mature ova of up to 0.8mm in all the mature stages of *P. imperator* (stage III-VI). It is therefore possible that the ova present in the ovary are regenerated at once some are released. Therefore, they 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 spawn approximately 126,000 eggs during a 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  $79,400 \pm 9,200$ . Fecundity is a specific reproductive trait and is adapted to the life cycle conditions 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. So, fecundity of *P. imperator* is the same order of magnitude in the present study and as well in an earlier study for the same species.

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 maximum proportion of fishes with mature gonads of *P. aucuatus* 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 absence of the fast moving East African Equatorial Currents that are common during the South East Monsoon period (June-July). Therefore, favorable environment for the survival of eggs, larval and post larval stages of this species seem to be the main drivers in spawning seasonality.

Mature individuals were observed in all months during the study period. This may suggest that *P. imperator* spawns continuously. Generally, larger proportion of mature individuals was recorded between March and April suggesting that this period was the peak of the spawning season.

The value of  $b$  (3.038) was close to the theoretical value ( $b = 3$ ) indicating isometric growth, occurring at the same rate for both length and weight so that its shape is consistent throughout development. The parameters of 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). This finding is in agreement with the general principle of fish growth. For Length-weight relationship, allometric coefficients ( $b$ ) estimates for both male and female *P. imperator* during the study were within the accepted range of 2.5 and 3.5 for isometric growth pattern (Froese and Pauly, 2006). The differences in  $b$  values can be attributed to the combination of one or more factors including habitat, area, season, stomach fullness, gonadal condition, sex, health, preservation methods and differences in the size of the specimens caught (Hossain *et al.*, 2011), and not all of these factors were accounted for in this study.

The condition factor of *P. imperator* did not vary significantly throughout the study period and may not be a good indicator of breeding pattern. It is probable that lack of variation in the condition of the fish may be attributed to a combination of factors e.g. conversion of energy to spawning activity or the availability of food in the fishing grounds among other factors which the present study did not account for.

## CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

This study established that *P. imperator* can be categorized as sexually dimorphic and gonochoristic. Females are on average larger than males and more vulnerable to overfishing. This population structure will lead to recruitment overfishing when a higher proportion of large-growing females are removed by the artisanal fishery before they have a chance to participate in reproduction. The persistent removal of immature *P. imperator* populations by the aquarium fishery below the sizes at maturity will expose them to growth overfishing, whereby the removal of under size at maturity of 25 cm and 28 cm for females and males respectively lowers the yield per recruit. Larger females spawn greater proportions of oocytes suggesting that sizes of female *P. imperator* can be used to generate egg production per female recruit and this can be used to illustrate potential effects of management measures on depleted stocks of this species. The poly-modal oocytes diameter distribution indicates that oocytes development in the ovary of *P. imperator* is asynchronous. GSI together with the proportion of mature individuals of *P. imperator* in all the sampled months has been found by the present study to have a prolonged spawning period with peaks in March to April. The relationship between total length and body weight show that *P. imperator* exhibits isometric growth pattern. The variation in relative condition factor,  $K_n$  may not be attributed to spawning activity but a combination of factors such as conversion of energy to spawning activity, or the availability of food in the fishing grounds among other factors.

## **6. 2 Recommendations**

Management of *P. imperator* should include measures based on parameters established in this study including its size variations between the sexes and size at maturity and fecundity. These parameters will help develop optimal size limits and harvest rates that optimize long-term, sustainable yields and protect the stock reproductive potential.

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**APPENDICES**

Appendix 1: Data sampling instrument/ form used in the present study

Obota Clay:  
University

M.Sc. Fisheries-Pwani

Reproductive biology of exploited populations of Emperor Angelfish, *Pomacanthus imperator* along the Kenyan Coast

	Total length (cm)	Standard Length (cm)	Body weight (g)	Gutted weight (g)	Gonad weight (g)	Average # of eggs in sample	Fecundity (Total # of eggs)	Mean egg diameter (mm)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								

Comments: \_\_\_\_\_

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