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Ovarian Development of the Penaeid Shrimp *Penaeus Indicus* (Decapoda): A Case for the Indian Ocean Coastal Waters of Kilifi Creek, Kenya

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Abstract

The Indian prawn *Penaeus indicus*, is one of the major commercial shrimp species globally. It is widely distributed in the Indo-West Pacific; from eastern and south-eastern Africa, through India, Malaysia and Indonesia to southern China and northern Australia. The species has been recorded to reach 22 cm, inhabiting depths of 100 m. Globally *P. indicus* is widely studied, although majority of the studies have focused on developmental stages between shallow waters and the deep seas. Studies indicate that development takes place in the sea before the larvae move into estuaries to grow, then return as sub-adults. However, studies on the maturity of this species in shallow waters and especially creeks and embayments are clearly lacking for the Kenyan coastline. This study was conducted in the Kilifi Creek, north coast Kenya, from the mouth at the Kilifi Bridge to past Kibokoni, some 5 km into the creek. Samples were collected from 6 landing sites. Morphometric and biological data including total length (TL, cm), carapace length (CL, cm), body weight (BW, g) and sex were recorded, and the specimens dissected to check for ovarian development and maturity. Ovarian development stages were determined from size, shape and colour of the ovaries, and through frequency analysis of the cortical granules. A total of 1,149 specimens were sampled from January to April, 2018. The catch mostly comprised of the 1.5 cm to 2.5 cm CL size class. However some individuals in the >4.0 cm CL size class were also recorded suggesting that some individuals still matured in the creeks although majority are known to migrate deep sea. Regression analysis showed a strong positive correlation between CL, and TL (r^2=0.7548) and BW (r^2=0.7497).The condition factor ranged from 0.19-0.94 indicating presence of both immature/spent (4.7%) and ripe individuals (95.3%) in good condition. Size frequency analysis of the ovary cortical granules displayed a poly-modal pattern with 2 peaks of immaturity and 3 peaks for developing and near-ripe respectively. The presence of spent stages as well as mature specimens in the creek waters indicated that apart from using these habitats as nursery and feeding grounds, some of the individuals might also be growing, maturing and spawning in the creek, calling for further studies into the assessment of the growth patterns and reproductive aspects of this species in the Kilifi Creek and other coastal waters.

Keywords: Indo-West Pacific; Kenya; *P. indicus*; Ovarian development

Introduction

Penaeid shrimps are the most important economic resources in world's crustacean fishing industry accounting to more than half of shrimp production [1] and hence forming an important component of worldwide fisheries [2]. Over the last three decades, numerous studies around the world have been conducted on penaeid shrimp stocks in efforts towards improving its sustainable management [3]. However, exploitation and sustainable management of these resources calls for a better understanding of their reproductive biology [4].

The Indian prawn *Panaeus indicus* is a small penaeid shrimp found in the Indo-West Pacific from Eastern and South-eastern Africa, through India, Malaysia and Indonesia to Southern Australia [5]. *P. indicus* are non-burrowing shrimps and are thus found mostly in sandy mud bottom; they have been reported to mostly inhabit shallow coastal waters with less than 50 m depth [6]. The shrimps are exploited by small-scale fishermen throughout the year along the creeks and hence serve as an important source of food protein for poor coastal communities as well as sources of income supporting the coastal village economies. Furthermore, the shrimp fisheries make crucial contribution to the domestic market and export commodities to other countries.

Along the coastal waters of Kenya, several studies have been conducted on shrimps and prawns, including studies on distribution of some deep water prawn and lobster species in Kenya's waters [7], stock assessment and population dynamics of penaeid prawns and managing the prawn fishery in Kenya [8], resource use conflicts especially in the Malindi-Ungwana Bay [9], impact of the shrimp trawling in Ungwana Bay on marine resources [10], preliminary investigations on the ichthyo-diversity of the Kilifi creek, Kenya [11] and more recently, analysis of the fish species composition and distribution in Kilifi creek [12]. However, in most of the coastal waters and embayments in Kenya, including the Kilifi creek, detailed studies on the biology of the Indian prawn *P. indicus* as well as other penaeid shrimps are evidently lacking. Consequently, lack of adequate information and data on the reproduction, spawning seasons, maturation and related biological attributes of the penaeid shrimps including the commonly exploited *P. indicus* remains scant and thus limits formulation of sound management strategies for these important fisheries. This study provides the first detailed presentation of the ovarian development, length-length and length-weight relationships, sex rations and body condition factor of *P. indicus* in Kilifi creek, Kenya.

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Numerous studies have been conducted on the ovarian development of shrimps in the world. A study to investigate the reproductive cycle and size at first maturity of *Penaeus schmitti* revealed occurrence of vitellogenic oocytes in the spent stage, indicating a rapid ovarian maturation after spawning [13]. Ovarian development of the wild pink prawn *Farfantepenaeus paulensis* in northern coast of Santa Catarian State Brazil revealed considerable differences in shape and colour of the ovaries in the wild. Four gonadal stages were observed according to the histological and macroscopic analysis performed; Immature, developing, ripe and spent [14]. Therefore the adoption of using four developmental stages in ovary identification for *F. paulensis* because of the absence in histological differences observed allowed the use of not more than four stages. Polymodal distribution of oocytes size as well as ripe cells remaining in ovary after spawning provide another evidence to conclude that a species performs partial spawning as a reproductive strategy in the wild.

A study on the ovarian development of the deep-water mud shrimp *Solenocera melantho* revealed four developmental stages: quiescent, developing, early ripe and ripe [15]. Gonadosomatic index analysis revealed that changes in shape and colour of the ovaries matched very well with the development of oocytes and the weight of the ovary. Colour change with ovarian development is highly known for decapod crustaceans and majorly in the family of penaeid shrimps [16]. Sometimes the maturation of penaeid shrimps is considered to have five stages which include the "spent stage" [17] but none of the specimens in the current study showed any sign of the spent stage. A study in the north-western part of the Persian Gulf showed a probable spent ovary which indicated that spawning is very rapid and females quickly die after spawning in this species or move to deeper waters [18].

A histological study of ovarian development of the Giant Red shrimp *Aristaeomorpha foliciae* (Decapoda) from the Southern Tyrrhenian Sea in Mediterranean revealed six ovarian maturation stages [19] namely: oogonia (OO) for stage one, early primary oocytes (EP) for stage two, late primary oocytes (LP) for stage three, early vitellogenic oocyte (EV) for stage four, late vitellogenic oocyte (LV) for stage five and postvitellogenic oocytes (PV) for stage six. The presence of spent stage in the penaeid species has led to a debate which came to a conclusion that maybe females may die after spawning or move to deeper waters deeper than 600 m where they will be missed by trawlers [20].

Understanding length-weight relationship (LWR) and Relative Condition Factor of crustaceans is essential in fisheries sector. LWR plays an important role in their stock assessment by providing important information on the habitat where the species lives [21]. LWR estimates condition factor of the fish which is an important indicator of physiological state of the species to its welfare [22]. It is important in monitoring feeding intensity, age and growth rates [23]. On the other hand [24] noted that condition factor is important in understanding the life-cycle of these species and therefore, it contributes towards management of the species.

Problem statement and justification of study

Several studies of different species have been done along the coastal waters of Kilifi creek for example fish species composition and distribution as well as some preliminary investigations on the ichthyo-diversity of the creek. However, little has been done on crustaceans and especially on reproductive studies of *P. indicus*. In response to this, this present study sort to investigate and describe the ovarian development of *P. indicus* in Kilifi creek, along the Kenyan coast, in relation to length-length and length-weight relationships and their sex ratios.

Aims and objectives

The overall objective of the present study was to assess the ovarian development of the Indian prawn *P. indicus* (*Decapoda*, *Penaeidae*) from the small-scale fisheries of the coastal waters of Kilifi Creek, Kenya.

The specific objectives of the study were to;

- Determine length-length (TL-CL), length-weight (CL-BW) relationships and Condition factor in the *P. indicus* population from the Kilifi Creek shrimp fishery.
- Determine length-frequency distribution and population pattern of *P. indicus* from the Kilifi Creek fishery
- Determine sex ratios of the *P. indicus* in the Kilifi Creek shrimp fishery population.

Materials and Methods

Description of the sampling site

This study was conducted in the Kilifi creek, north-coast Kenya, from the mouth of the Kilifi bridge to past Kibokoni fisheries landing site stretching about 5km into the creek (Latitude 3° 36′ S; Longitude 39° 51′ E) as shown in Figure 1. The fishing grounds consolidated in the Kibokoni fish landing site included Kidundu, Kuchi, Shangashani, Mtongani, Mzombere, Maringoni and Matope which stretch from the creek mouth to the end of the creek in a SE-NW direction.

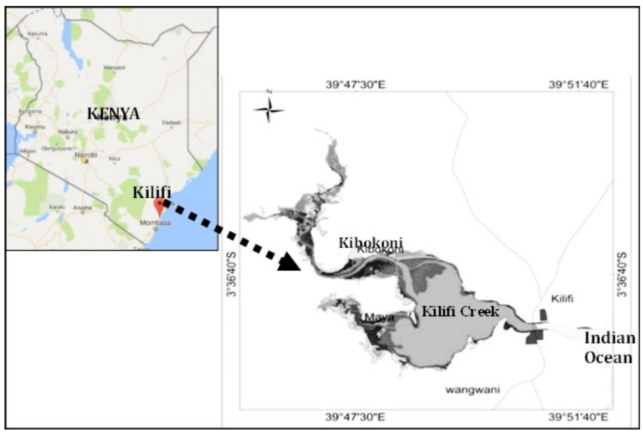

Figure 1: A map of Kenya showing Kilifi Creek and the location of the sampling site at Kibokoni.

Field sampling and sample analysis

Sampling was conducted once a month during the spring tide when most fishers targeted the penaeid shrimps in the creek. Samples for *P. indicus* were collected during early mornings when the shrimp fishers landed their catches at the Kibokoni landing site. The fishing gears used by the fishers ranged from traditional traps to modern nets including monofilament gillnets and prawn seines fabricated from small-sized mesh nets. The samples were immediately chilled in ice

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and fixed with 10% formalin upon arrival in the laboratory pending analysis.

The preserved specimens were sexed based on the presence of thelycum for females and petasma for males [25]. Thereafter, all the specimens were measured for total length (TL); from the tip of the rostrum to the tip of the telson, and carapace length (CL); from the mid-dorsal posterior edge of the carapace to the tip of the rostrum to an accuracy of 0.01 cm using digital slide calipers [26]. Body weight (TBW) was taken on an electric balance (Shimadzu, EB-430DW) at a precision of 0.01 g accuracy. Sex ratio analysis were calculated for each size-class and sampled during the study period, and variations from the expected ratio of 1:1 tested using a Chi-square test based on the monthly samples data.

Length-weight relationships were analysed using regressions of the Carapace length (mm) against Body weight (BW, g) following the [27] by the power function, W=aL^b. The parameters were fitted to a regression line by the least square method and the body condition factor (K) for each specimen calculated using Fulton's formula, K=100×(W/L)^3. All statistical analyses were conducted using data analysis add-ins in Microsoft Excel® 2013. Statistical tests were considered significant at 95% confidence level (α=0.05)

Maturity stage analysis was conducted using the keys adapted from as shown in Table 1 and Figure 2. The specimens were dissected and size , shape and cortical granules of the ovaries noted to determine the stages of maturity for each individual (Figure 3).

Ovary	General Appearance	Colour	Oocyte Development
Stage 1 (immature)	Tubular, posteriorly divided into two parallel branches and extending to 2nd abdominal somite.	Translucent and colourless	Resting oocytes-100%
Stage 2 (early developing)	Slightly swollen, frontal part branched into two, median part subdivided into 8-10 lateral lobes, caudal lobes extending to 3rd abdominal somite.	Light-orange or light grey, semi-translucent	Resting oocytes-30%
			Developing -70%
Stage 3 (nearly ripe)	Expanded, frontal lobes covering about half of lateral stomach, lateral lobes covering half of dorsal hapato-pancreas, caudal lobes extending to 4th abdominal somite.	Bluish grey	Resting oocytes-10%
			Developing oocytes-3%
			Expanding oocytes-45%
			Mature oocytes-10%
Stage 4 (Ripe)	Greatly swollen, frontal lobes completely covering lateral stomach, lateral lobes covering almost entire dorsal hepato-pancreas, caudal lobes as a pair of inflated tubes and extending to 4th abdominal somite	Pale black	Resting oocytes-5%
			Developing oocytes-15%
			Expanding oocytes-30%
			Mature oocytes-50

Table 1: Criteria used for determination of ovarian maturity stages of penaeid shrimps.

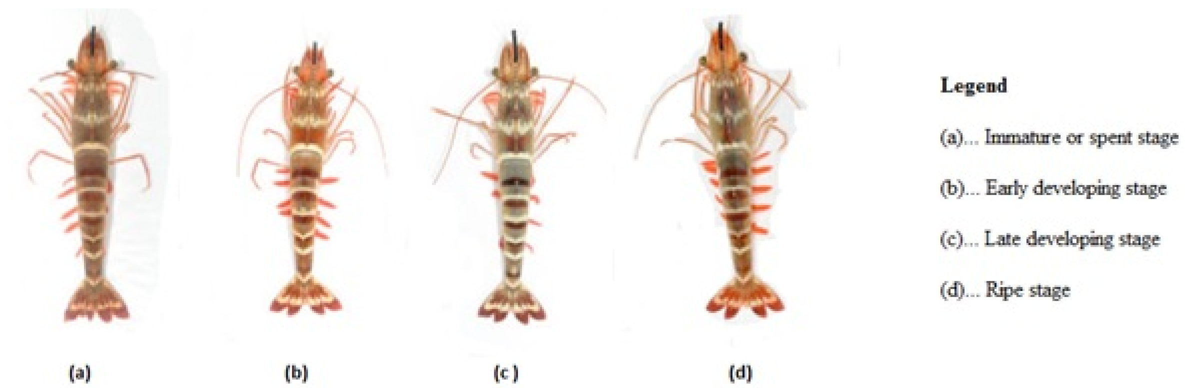

Figure 2: Ovarian development stages based on appearance of ovarian mass at different stages of maturity used in the present study on *P. indicus* in Kilifi Creek.

The reproductive biology of the samples was studied by correlating their macroscopic and microscopic characteristics and the gonadal developmental stages. The five ovarian maturation stages were recognized from variations in colour, as well as development and arrangements of cells. These stages were designated as immature (translucent), early developing (yellow), late developing (light green), ripe (green) and spent (white/creamy). The present study showed that oogonia cells found throughout the ovarian development though they were predominated in the undeveloped and spent stages. In the immature stage, oogonia were found as clustered in a well-defined area of the ovarian wall along the gonad, known as the "zone of proliferation" (Figure 2a). This type of ovary was found in only young and immature shrimps. Whereas yolkless oocytes cells without nuclear membrane were observed during the early developing (Figure 2b) and late developing (Figure 2c) stages with small number of oogonia and yolky oocyte cells with clear nuclear membrane were found in nearly ripe and well organized in the ripe stage (Figure 2d). This stage was believed to be the last stage of maturity before spawning. Ripe ovaries were characterized by the presence of fully mature oocytes. A typical distinctive feature of this stage was the cytoplasm of mature oocytes shows conspicuous cortical rod at the cell periphery. After spawning the ovary was designated as spent, it was observed that ovarian organization disappears, leaving holes left by the released oocytes, as well as remains of disintegrating mature oocytes.

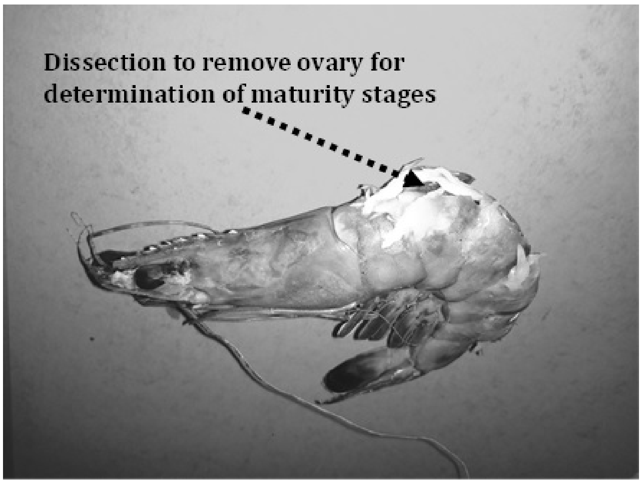

Figure 3: Dissection of a specimen of the Indian prawn (*P. indicus*) sampled from Kilifi Creek, Kenya for analysis of ovarian development.

Results and Discussion

A total of 1148 specimens were collected from the Kilifi Creek small-scale fisheries in the present study for determination of length-length and length-weight relationships, sex rations, condition factor, and ovarian development in *P. indicus* from the small-scale fisheries in the creek Figure 3.

Figure 4: A sample of the Indian prawn (*P. indicus*) specimens from Kilifi Creek, Kenya.

Analysis of the 1148 *P. indicus* specimens collected from Kilifi creek, Kenya during the study period January through April, 2018, showed 612 females (53%) and 536 males (47% males) giving an overall sex-ratio of 1:1.4. Chi-square Tabular=4.06, therefore the sex ratios did not differ from the expected 1:1 ratio (Stat=10.82, p=0.14).

Considerable differences in colour, size and shape of the ovaries was observed. In this study, three distinct ovarian stages were recognized based on the marked differences in colour, shape, size and cortical granules of the ovaries: Stage-1, Immature/Spent; Stage-2, early developing and Stage-3, Late developing stages. Size variations (TL, CL and BW) were noted for the samples with maturity and sex, and the values increased with the development of ovaries through the developmental stages. Body sizes of the samples collected during this study in January through April 2017 varied from 3.5 cm to 16.1 cm TL, 0.2 cm to 4.6 cm CL and 0.524 g to 39.482 g BW.

Length-frequency analysis showed that the samples from the Kilifi Creek shrimp fishery were dominated by the 1.5 cm to 2.0 cm CL size class, accounting for 88.9% (1021 out of the 1149 of the total specimens) samples. The 2.0 cm to 2.5 cm CL size-class accounted for 7.7% of the total specimens while fewer and fewer individuals were recorded in the 2.5-3.0, 3.0-3.5 and subsequent classes. However, some individuals were also recorded in the >4 cm size-class (0.4%) an indication that the creek was also inhabited by bigger sized *P. indicus* individuals (Figure 3).

The condition factor K of the sampled catch from the Kilifi Creek shrimp fishery ranged from 0.19 to 0.94 indicating very high variations in the body condition of the *P. indicus* in the creek, which can be linked to the variations in the spawning and maturity status of the individuals; with Stage, 1: immature/spent (4.7%) having low K values compared to latter stages Stage 3, Late maturing, and Stage-4, Ripe individuals which accounted for 95.3% of the individuals sampled.

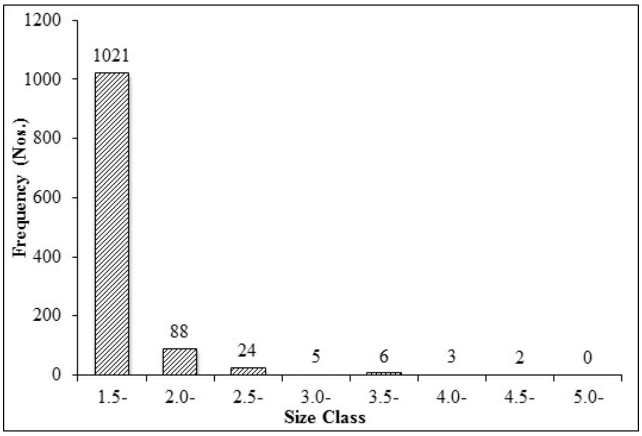

Figure 5: Size frequency analysis for the *P. indicus* specimens from the Kilifi Creek shrimp fishery during January-April, 2017.

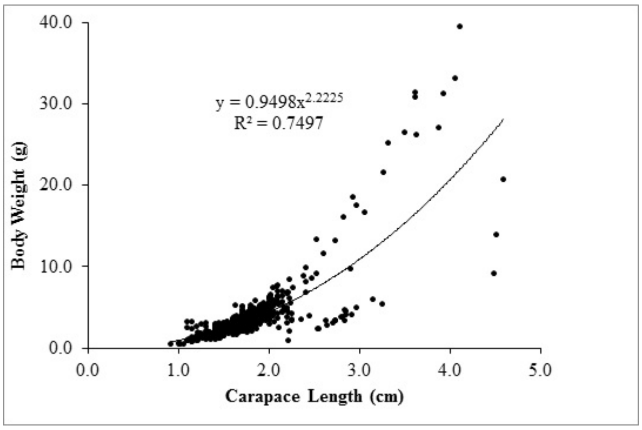

Figure 7: Length-Weight relationship (CL-BW) in *P. indicus* specimens from Kilifi Creek fishery during January-April, 2017.

Regression analysis for length-length (LLR), and length-weight (LWR) relationships showed a strong positive correlation between Total length TL and Carapace length CL (r^2=0.7548), and Carapace length CL and Body Weight BW (r^2=0.7497) as shown in Figures 4 and 5.

Tesch indicated that both LLR and LWR in shrimps can be affected by several factors including habitat type, area, season, stomach fullness, gonad maturity, sex, body condition, preservation techniques and length ranges of collected specimens. However, even though most of these factors were not studied in the present study, the results of the condition factor K and LLR/LWR suggest healthy/normal development for the *P. indicus* population in Kilifi Creek fishery.

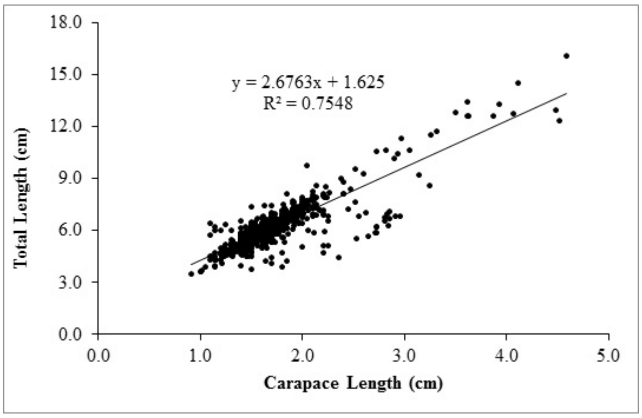

Figure 6: Length-Length relationship (CL-TL) in *P. indicus* specimens from Kilifi Creek shrimp fishery during January-April, 2017.

In the present study, Stage-1 (immature/spent) was recorded in all the length classes with varying proportions to the other maturity stages across the length classes, with a fairly standard normal distribution in the population of the *P. indicus* from the Kilifi Creek fisheries. This stage (Stage-1) was dominant in the 3.5 cm to 4.0 cm length-classes while the rest of the individuals in this class were categorized into Stage 3 (Late-developing) suggesting that this length classes comprised of mostly the spawning, spent and late developing individuals. This is further evidenced by the presence of Stage 1 individuals in >4 cm Length-class, comprising of mainly the "Spent" or post-spawn individuals. This is also an indication that the *P. indicus* species was utilizing the Kilifi Creek habitats as spawning grounds, and not just nursery and feeding grounds for the young stages of the shrimp. On the other hand, Stage 2 (Early developing) specimens dominated in 2.0 cm to 2.5 cm and 4.5 cm to 5.0 cm length classes comprising of mostly the early developing individuals for first-time spawners and second/subsequent season spawners after the "spent" stage (Figure 6).

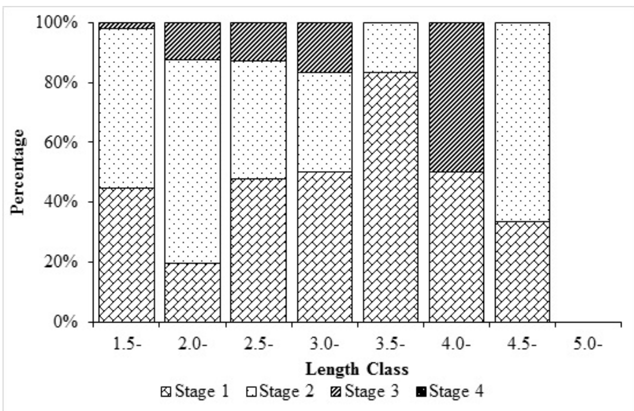

Figure 8: Length-frequency distribution and maturity developmental stages of *P. indicus* samples from the Kilifi Creek fishery during January-April, 2017.

During the present survey from January-April, no ripe-individuals were recorded suggesting that the spawning season for the *P. indicus* in

Kilifi Creek occurred during the other months of the year, or the spawning grounds were likely located in the deeper areas of the creek which were inaccessible to the foot fishers who concentrated mostly on the low-water edges of the creek (pers. observ.)

Size frequency analysis of the length classes displayed a poly-modal pattern with three major peaks between 1.34-1.42, 1.48-1.53 and 1.60-1.63 length classes as shown in Figures 7-9.

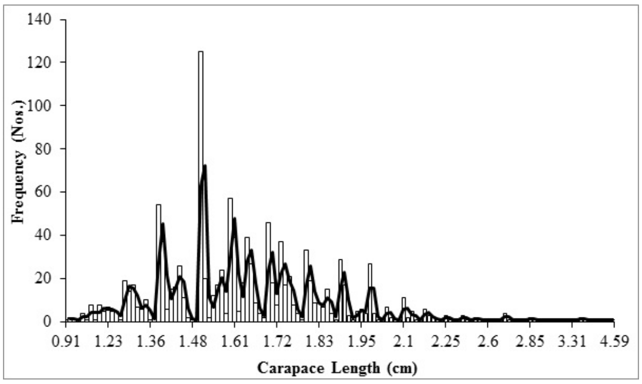

Figure 9: Length-frequency of *P. indicus* samples from the Kilifi Creek fisheries showing a polymodal distribution pattern in the population.

Considerable differences in colour, shape, size and cortical granules of ovaries at different developmental stages were observed in the present analysis of *P. indicus* in Kilifi Creek, similar to earlier studies on penaeid shrimps in the indo-pacific region [4,16]. However, in the present study, only three developmental stages were recorded; immature/Spent, Early developing and Late Developing/Nearly ripe. Dall et al. [16] indicated that ovarian development in shrimps is sometimes considered to have five stages including "spent stage". The probable absence of the five developmental stages in the samples of *P. indicus* from the Kilifi Creek is partly attributed to the short duration of sampling (4 months) in this study as well as due to the fact that the samples were obtained from the small-scale fisheries data where fishing gears are restricted to a certain minimum mesh size. A few individuals of length classes >4 cm CL size classes recorded in the study suggest the presence of "Spent" stages in the creek.

Results of the length-length analysis for Total length (TL) against Carapace length (CL) showed a significant correlation between the two parameters ($p<0.05$) indicating a healthy population of *P. indicus* in the Kilifi Creek as supported by the high condition factor (K=0.9) (Table 2).

ANOVA						
	df	**SS**	**MS**	**F**	**Significance F**	
Regression	1	123.8	123.8	3531.2	0	
Residual	1147	40.2	0.04	-	-	
Total	1148	164	-	-	-	
	Coefficients	**Standard Error**	**t Stat**	**p-value**	**Lower 95%**	**Upper 95%**
Intercept	0	0	-1.6	0.1	-0.12	0.01
TL (cm)	0.3	0	59.4	0	0.27	0.29

Table 2: Length-Length regression analysis for Total Length (TL) and Carapace Length (CL).

Further, length-weight analysis showed that there was a significant positive correlation between Carapace length CL and Body weight BW ($p<0.05$) (Table 3).

ANOVA						
	df	**SS**	**MS**	**F**	**Significance F**	
Regression	1	105.5	105.5	2068.2	0	
Residual	1147	58.51	0.05	-	-	
Total	1148	164.01	-	-	-	
	Coefficients	**Standard Error**	**t Stat**	**P-value**	**Lower 95%**	**Upper 95%**
Intercept	1.34	0.01	134.6	0	1.32	1.36

BW (g)	0.1	0	45.48	0	0.1	0.11

Table 3: Length-weight regression analysis for Carapace Length (CL, cm) against Body Weight (BW, g).

During the present survey, histological observations of ovaries showed that the smallest mature individual of the *P. indicus* in the Kilifi Creek shrimp fishery was 1.9 cm CL. Due the fishery regulations restrictions on the fishing gears used especially with regards to mesh size, the 1.9 cm CL could not be determined as the size at sexual maturity (L_m). However, majority of the mature individuals were recorded in the 2.0 cm to 2.5 cm CL size classes suggesting that size at massive maturity could be determined as L_{50}=2.0 cm CL. Caution should however be exercised when using the fishery dependent data as numerous factors, in addition to the mesh size restrictions would influence the accuracy of the determination. The size at sexual maturity is of special interest in fisheries management and is widely used as an indicator for minimum permissible capture size [29] and therefore detailed studies using experimental survey data should be conducted to determine the size at sexual maturity of the *P. indicus* population in Kilifi Creek. Furthermore, such studies would provide further insight into the specific combination of factors affecting size at sexual maturity in the populations of *P. indicus* in Kilifi Creek and other coastal waters of Kenya. Factors that may influence the size at maturity include in environmental factors, particularly water temperature, population densities and food availability.

Observation of cortical granules is considered as one of the most accurate methods for the determination of mature females [30]. In similar studies, the appearance of cortical crypts in the peripheral part of the cytoplasm in the oocytes is determined as a sign of spawning for some penaeid shrimps [31,32]. However, the development of visible cortical crypts might not be a perquisite to spawning in all the species. Therefore, analysis of cortical crypts in the cytoplasm of the oocytes of *P. indicus* was not determined in the present study, and the ovarian maturity stages were determined and categorized on the basis of ovary colour, shape, size and cortical granules diameter as a reliable method.

Conclusion and Recommendation

This study provides the first report of the some key attributes of the biology of *P. indicus* in Kilifi Creek, Kenya, including data and information on length-length and length-weight relationships, ovarian development and sexual maturity, sex ratios and condition factor for this important species which is useful for the sustainable management of the shrimp fisheries in the coastal waters of Kilifi.

The presence of spent stages as well as mature specimens in the creek waters indicates that the Indian prawn *P. indicus* in the coastal waters of Kilifi utilizes the Kilifi Creek habitats as nursery and feeding grounds, as well as some of the individuals growing, maturing and spawning in the creek. However, more detailed experimental surveys should be conducted to decipher this phenomenon, as well as provide data and information on the specific habitats utilized for spawning in order to design strategies for protection of such habitats.

The presence of mature specimens but no gravid females in the creek waters during January through April indicates that the *P. indicus* population does not spawn all year round but the spent individuals affirm the possibility of the species spawning in the creek, with limited migrations into the deeper waters of the Kilifi to spawn or migration out of the creek after spawning. However, more detailed studies covering the estimated longevity of 18 months for this species should be conducted in order to determine the spawning seasons, the size at sexual maturity and the spawning periodicity of this species in the creek, growth patterns and other reproductive aspects of the *P. indicus* population in Kilifi creek and other coastal waters.

External characteristics of the ovary, such as size and colour are closely related to the development and internal organization of the germ cells, and based on that data; size at maturity can be estimated. Therefore, detailed studies should be conducted on ovarian and sexual maturity for this species, estimation of the population attributes such as stock size, feeding habits etc. in order to fully guide the management strategies for the *P. indicus* fishery in Kilifi creek with regard to required regulations on fishing effort, mesh size restrictions, closed fishing areas and fishing grounds as well as impact of the shrimp fishery in terms of bycatch attributes for other species. Notwithstanding, this study provides a very important step in understanding the biology of the *P. indicus* population in Kilifi Creek for the management of the small scale fishery.

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