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GROWTH COEFFICIENT AND ASSESSMENT OF SPECIES SPECIFIC PRIMERS FOR AMPLIFICATION OF mtDNA OF THE ROYAL SPINY LOBSTER, *Panulirus regius* (De Brito Capello, 1864)

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ABSTRACT
The Royal Spiny Lobster, *Panulirus regius* (De Brito Capello, 1864) is one of the most important marine crustaceans in Nigeria. Pattern of growth, condition factors and the primers for amplification of the mitochondrial DNA of a Palinuridae lobster, *Panulirus regius* along Nigeria coastal water were investigated from January to June 2017. The specimens ranged in carapace length from 13.1 to 28.3 cm, weighing 53.1 to 195.6g. Length-weight relationship was estimated as $W = 0.0145L^{1.600}$ given an allometric growth model. The calculated maximum Fulton's Condition Factor (K_F) and Allometric Condition Factor (K_A) were 2.5 and 0.0003 respectively. The designed primers were defined in sequences, number of band and percentage polymorphic band. The amplification result showed high polymorphism level based on the banding patterns of the samples. With this species specific primers specimen can be identified using minimal quantities of different tissues with only DNA isolation, PCR and electrophoresis for varieties of biological researches. This study revealed that morphometric characterization coupled with molecular analyses could make proper classification of crustaceans in Nigeria possible to achieve.
Keywords: Condition factor, DNA, Lagos Harbour, lobster, primer, Nigeria

INTRODUCTION

The genus *Panulirus* is of interest to biologists because of its high level of biodiversity and its wide geographic dispersal, while it is also a good commodity in marine fisheries (Ptacek *et al*., 2001; Abdullah *et al*., 2010). *Panulirus regius* has been regarded as data deficient because of the little quantitative information on its population trends; however, anecdotal reports suggest it has been intensively fished for decades along much of the West African coast with little or no regulation (Clotilde-Ba *et al*., 1997). According to FAO (2001), this species is caught as by-catch by trawlers in Nigeria.

The Royal Spiny Lobster (Synonym: Green Lobster) inhabits shallow water about 55 m and mostly found between 5- 15 m on rocky bottoms (Holthuis 1991). This species can be easily recognized from true lobsters by the presence of phyllosom (unique larval phase), absence of claws on the first four pairs of legs and its long, thick, spiny antennae (Holthius, 1991). They have slightly compressed carapace and lack lateral ridges while the body plan follows that of all decapods and is made up of twenty-one (21) body segments. These segments can be grouped together into the cephalothorax (head and chest area) and abdomen (Pollock, 1995).

Scientific data about the presence of species in a specific area is fundamental ecological parameter and foundation for understanding population ecology (Moruf and Adekoya, 2018). This was why noninvasive genetic research method was developed, using samples with low DNA quantity as a research objective for identification of the species (Paden *et al*., 2009). Mitochondrial DNA (mtDNA) is present in all animal tissues, it has a small genome of a simple structure, no coding parts (introns) and it has different rates of evolution in all parts, which is important for solving philogenethic questions on different taxonomical levels (Zhang and Hewitt, 1996). mtDNA is a circular, two-stranded molecule which varies in size from 15 000 to 20 000 base pairs (bp). Control region is a non-coding part of the mtDNA, 1000 bp long with sequences that are acting in replication and transcription of the mitochondrial DNA (Odak, 2004). Primer being a short synthesized oligonucleotide is designed to recognize the precise sequence of DNA nucleotides, which is afterwards used as a model for PCR.

There are various studies on crustacean species in respect to morphometric, feeding habit and some aspect of reproductive biology as a separate entity (Lawal-Are 2003; Lawal-Are and Akinjogunla 2012; Moruf and Lawal-Are 2017a, Moruf and Ojetayo, 2017). Currently in Nigeria, no quantitative information to draft a management plan for marine crustaceans and until now, no known published information is available on the DNA analysis of the Royal Spiny Lobster in Nigeria. To bridge that gap, the goal of this work was to investigate the morphometric characters and to describe primer for amplification of control region of mtDNA for *Panulirus regius* found off Lagos Harbour. The result would ease ecological researches and management of this species.

MATERIALS AND METHODS

Description of Study Site

The sheltered parts of sea areas where ships and boats can berth to offload and take on goods are regarded as habour. The 2km wide harbour located in Lagos state, Nigeria, is geographically located at GPS co-ordinate of 6° 39' 16"N and 3° 40' 11" E with average depth of 7.5meters (Commodore Channel region) . It receives inland waters from Lagos Lagoon and Badagry Creek in the west and represents the only opening to the sea for all the Nigerian South-Western lagoons (Onyema, 2009; Onyema and Popoola, 2013). The sampling station lies along the eastern parts of the Lagos Harbour, the commodore channel (Fig. 1) which is at the mouth to the Atlantic Ocean having a tidal rhythm of semidiurnal.

Collection of Specimen

Samples were obtained from commercial trawl catches at the study site on monthly basis for six months (January to June 2017) and between 8.00 hrs and 12.00 hrs on each sampling day. A total of 132 Royal Spiny Lobsters (Fig. 2) was collected during the sample period. The specimens were identified using taxonomic keys of Schneider (1990) and immediately transported to the laboratory for analysis.

Determination of Morphometric Parameters

The carapace length and weight were measured using vernier caliper and sartorius weighing balance (Model: DT1001A) to nearest tenth of a centimetre and gram respectively. All measured data were recorded in a pro-forma sheet. The growth pattern of the specimens was estimated using the length-frequency distribution (Peterson's method). This method is based on the fact that the length of one age tends to form a normal distribution. The lengths of the lobsters sampled were plotted against their respective frequencies.

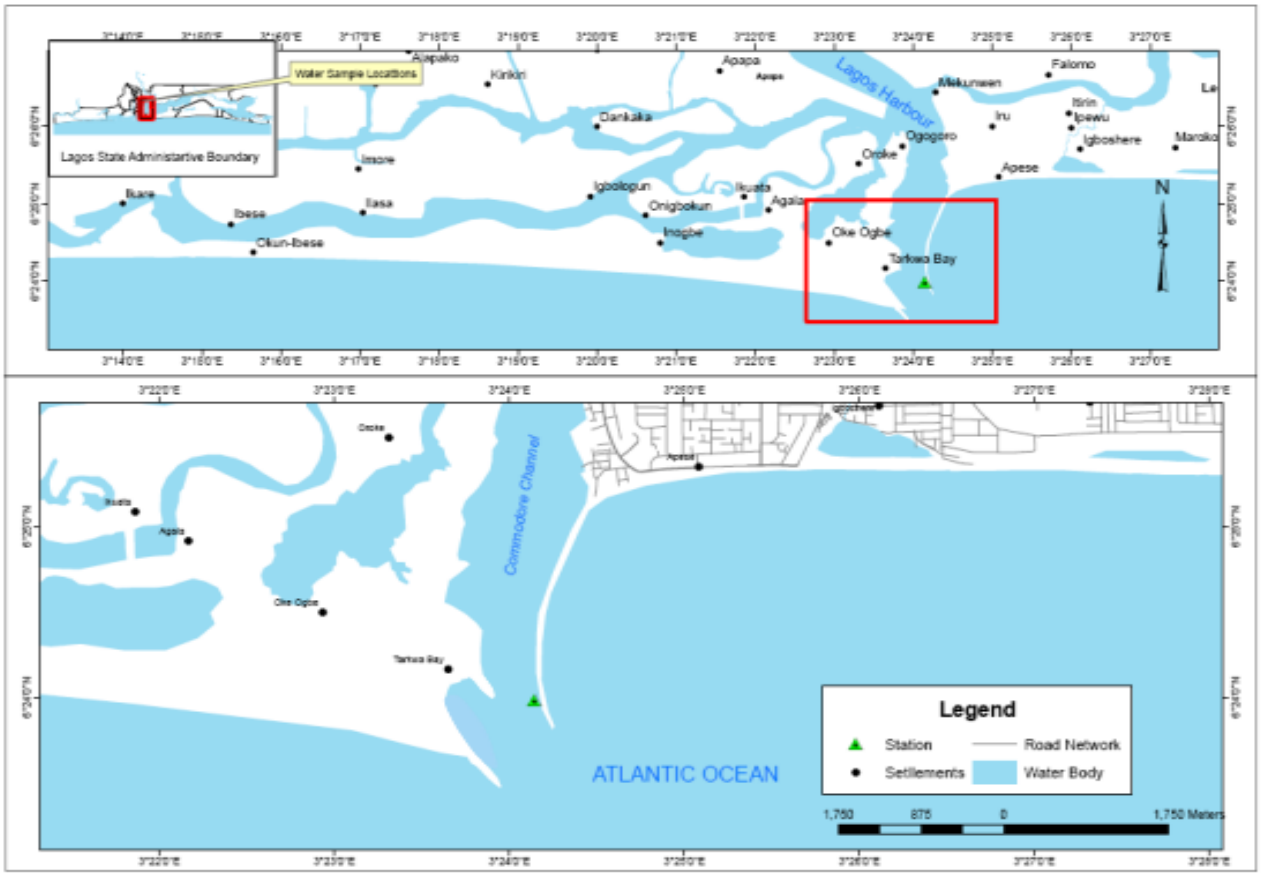

Fig. 1: Map of Lagos Habour area showing the sampling site

Figure 2: The Royal Spiny Lobster, *Panulirus regius* (De Brito Capello, 1864)

Carapace length-weight relationship of the samples is represented using the equation:

$W = aL^b$ …… 1 (Pauly, 1983)

W= Weight (g)
L= Carapace length (cm)
a= Regression constant (intercept)
b= Regression coefficient (slope)
Transformed logarithm relationship was:

$Log\ W = Log\ a + b\ Log\ L$ ……2 (Parsons, 1988)

Condition factors:
Fulton Condition Factor (K_F):

$KF = 100 \times (\frac{W}{L^3})$……..3 (Fulton, 1904)

Allometric Condition Factor (K_A):

$KA = \frac{W}{L^b}$…… 4 (Tesch, 1968)

Where w = Total body weight (g)
L = Carapace length (cm)
b = Regression coefficient (slope)

DNA Analysis

mtDNA were isolated from 100 mg of lobster's cheliped tissues preserved in 99.9% absolute ethanol using Norgen cells and tissue genomic DNA isolation kit from Norgen Biotek Corporation (Canada). The extracted DNA samples were amplified at a commercial company, Inqaba (Nigeria) for PCR analysis For development of primers, sequences of mtDNA of mentioned animal species from the internet gene database - GenBank were used according to Innis and Gelfand (1990). The protocol involved 7µL distilled PCR grade water, 2. 1µL of Primer F and 1µL of Primer R, 10µL PCR master mix (dNTPs, buffer, $MgCl_2$ and Taq polymerase) and 1µL gDNA. PCR parameters are as follows: Denaturation in 95°C (30s) repeated for 45 cycle, annealing in 50°C (30s) repeated for 45 cycles, elongation at 72°C (1 min) repeated for 45 cycles and another elongation at 72°C (10min).

The extracted DNA samples were amplified using Cytochrome Oxidase sub-unit 1 (CO1) according to the method of Paden *et al.* (2009). The 5' end of cytochrome c oxidase sub unit I gene region was amplified using the primer pair LCO1490 5'-GGT CAA CAA CAA ATC ATA AAG ATA TTG G-3' and HCO2198 5'-GGT CAA CAA CAA ATC ATA AAG ATA TTG G-3'.

Microsoft Excel 2010 and SPSS software (2015 version) were used to analyze data.

RESULT

Morphometric Characters and Condition Factors of *Panulirus regius*

At the end of the field work, 132 samples of *P. regius* were studied. In the carapace length frequency distribution of the lobster, size class 13.5 - 14.4 cm has the highest abundance with species exhibiting unimodal size distribution (Fig. 3). *P. regius* has a range of 13.1 - 28.3 cm in carapace length and 53.1- 195.6g in total weight as illustrated by the Log length/Log weight relationships (Fig. 4). The carapace length and body weight of the lobsters (with r value of 0.919) were highly correlated. The lobster exhibited negative allometric growth as shown by the mean exponents 'b'=1.600.

The variations in condition factors of *P. regius* by size group are presented in Table 1. The highest K-values were recorded for small size group (12.5-17.4 cm) and subsequently decreased with increase in size of the specimen.

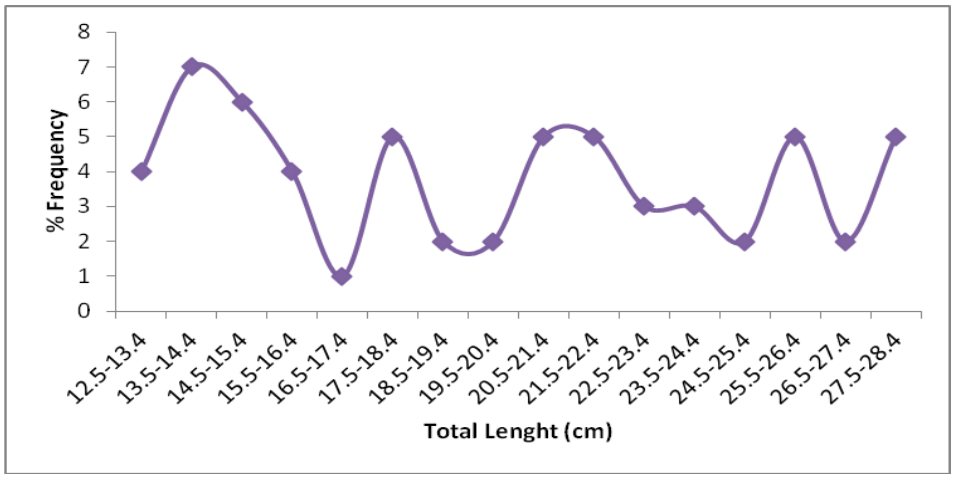

Fig. 3: Length frequency distribution of the Royal Spiny Lobster *P. regius* (Jan. – June, 2017)

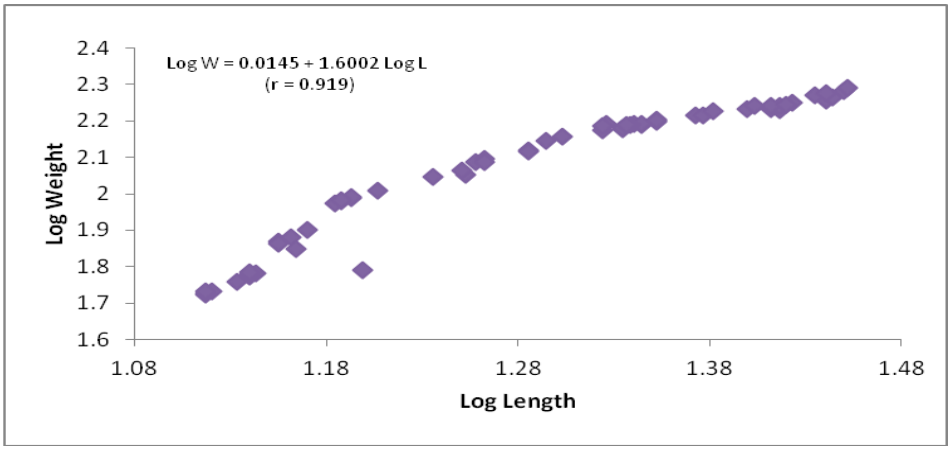

Fig. 4: Log length- Log weight relationship of *P. regius* (Jan. – June, 2017)

Table 1: Condition factors for the Royal Spiny Lobster, *P. regius* (January – June, 2017)

Size	Interval	N	CL(cm)	WT(g)	K_F	K_A
Small	12.5 - 17.4	48	15	84	2.5	0.0003
Medium	17.5 - 22.4	41	20	140	1.8	0.0002
Large	22.5 - 28.4	43	25	173	1.1	0.00009

DNA Amplification

Using computer programs and obeying rules for primer design, we have created primers for amplification of the mtDNA control region of *Panulirus regius*. The DNA was size separated on 1% agarose gel and viewed under the ultraviolet trans-illuminator for DNA quality and yield assessments. A number of distinct DNA fragments for each primer are shown in Table 2. Nineteen (19) bands amplified by two different primers, were scored among the accessions. Fifteen (15) of these nineteen (19) bands were highly polymorphic with percentage polymorphism put at 78.9%. The numbers of amplification products were 9 and 10 with primer HCO2198 producing the minimum number of (9) band and average of 9.5 bands was obtained. DNA profiles produced by both primers are shown in Plates 2 and 3.

Table 2: Specific primers for *Panulirus regius* with the sequence and the characteristics of amplification products

	LCO1490	HCO2198	Total	Average
Primer sequence (5'-3')	5'-GGT CAA CAA CAA ATC ATA AAG ATA TTG G-3'	5'-GGT CAA CAA CAA ATC ATA AAG ATA TTG G-3'		
Number of bands	10	9	19	9.5
Polymorphic bands	8	7	15	7.5
Percentage Polymorphic bands (%)	80	77.8	78.9	

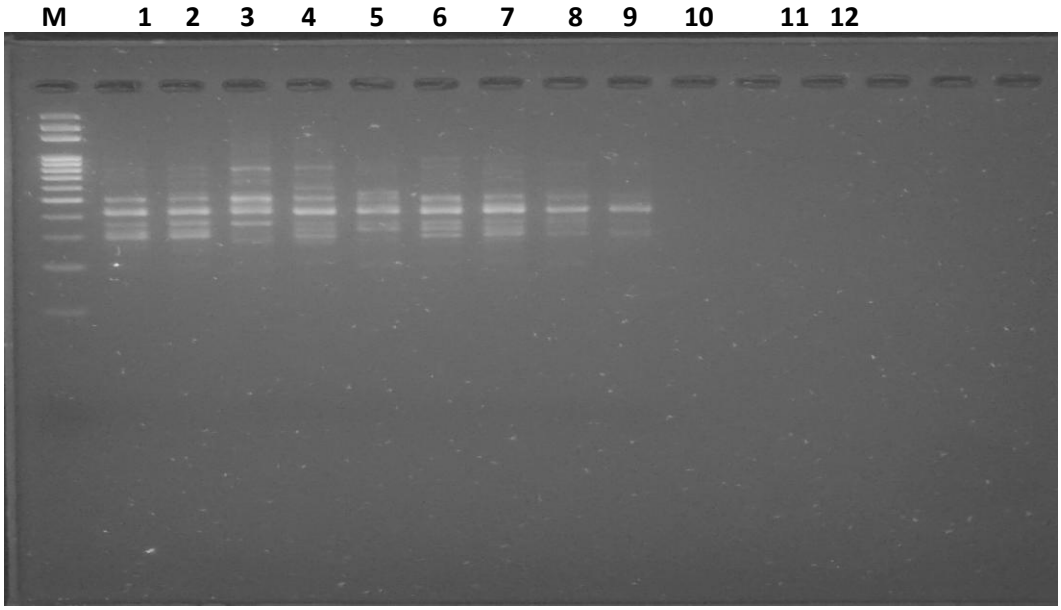

Plate 2: DNA profile produced by primer LCO1490. M represents the 100 bp DNA ladder which serves as the reference point; 1 to 12 corresponds to bands produced by the amplified DNA from the 12 *Panulirus regius* samples.

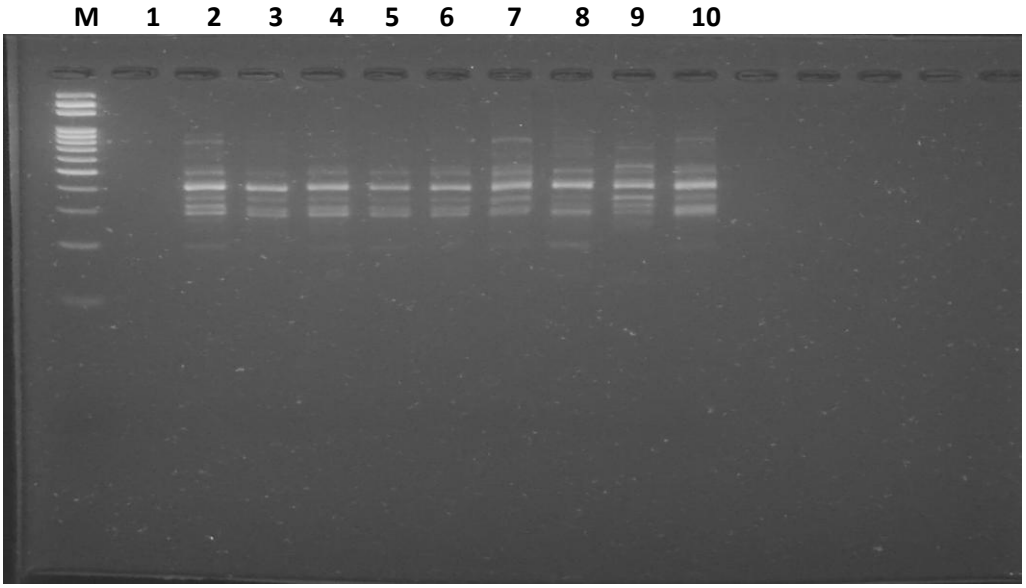

Plate 2: DNA profile produced by primer HCO2198. M represents the 100 bp DNA ladder which serves as the reference point; 1 to 4 corresponds to bands produced by the amplified DNA from the 12 *Panulirus regius* samples.

DISCUSSION

The sample size clearly indicates the low frequency of the Royal Spiny Lobster in the harbour, an indication that it is a marine species which only comes to harbour for food or reproduction. Unfortunately, no published ecological data on *P. regius* in Nigeria. The largest size with a total length of 28.3 cm while the smallest *P. regius* of 13.1cm total length were recorded in this study. The size group of 13.5-14.4 cm occurred most. This result was in agreement with Freitas *et al*., (2007) on lobster species from Cape Verde with males attaining larger size than females. In the work of Dineshbabu (2008), *Nephropsis stewarti* can reach a maximum length of 160 cm. This implies that *P. regius* in Lagos Harbour may be a smaller size species among spiny lobsters.

The total weight ranged between 53.1 to 195.6g with *b* value of 1.600 showing a negative allometric growth. The correlation co-efficient (r = 0.919) indicated a positive correlation between weight and length, the implication that increase in length gave a corresponding weight increase. This conforms to the work of Vaitheeswaran *et al*. (2012) on *Panulirus versicolor* along India coast. The condition factors (K_F and K_A) for *P. regius* varied in relation to size, decreasing with increase in size. Same inverse proportionality has been reported for another marine crustaceans, *Portunus validus* by Moruf and Lawal-Are (2017b). In studies of population dynamics, high "k" values of a crustacean shows adaptable environmental conditions, hence, it can be suggested that the study sites is a favourable environment for the Royal Spiny Lobster, *Panulirus regius*.

Noninvasive methods for identification of animal species are mostly based on detection of DNA sequences, using sequencing and phylogenetic analysis (Farrel *et al*., 2000). Based on the designed primers we should be able to identify animal species from samples using only DNA isolation, PCR and electrophoresis while more expensive and longer sequencing can be avoided (Paden *et al*., 2009). The present investigation gave useful information on the primers, DNA

amplification and hint on diversity of *P. regius*. The level of polymorphism observed in the study indicated a fairly wide and diverse genetic base. This observation conforms to the results of Moh *et al*., (2013) on the verification of the genus *Thalassina* using molecular and morphological characters.

By designing species specific primers we wanted to ease and accelerate identification of animal species present in Nigerian coast, while using sample acquired by noninvasive methods and samples with low DNA quantity. These primers can be used where the subject of the research is small quantity of tissue in which the DNA degradation has occurred. They are specific for the Royal Spiny Lobster, *P. regius* and are important in evolutionary research, conservation and shellfish management. This is a baseline study for further researches which will contribute to effective management and conservation of aquatic resources.

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