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Effect of Seasonal Fluctuations on the Nutrient Content of Whole Body of *Callinectes pallidus*

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Authors' contributions

This work was carried out in collaboration among all authors. Author OAO designed the study, Author OBA sourced for the samples while Authors NUA, GON and UU managed the analyses. Authors OAO and AAB wrote the first draft of the manuscript. All Authors read and approved the final manuscript.

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ABSTRACT

Due to the effect of species, age, sex, environment and season on the nutrientcomposition of seafood, the effect of seasonal fluctuations on the nutrient content of whole body of *Callinectes pallidus* caught during dry and wet seasonswas investigated.The proximate composition of the whole body of *Callinectes pallidus* caught in the month of February (dry season) and June (rainy season)was determined using standard methods, the fatty acid composition was analysed using Gas Chromatography- Mass Spectrophotometer and the amino acid composition was analyzed using Amino Acid Analyzer. Parameters such as crude fat, ash content and carbohydrateshowed significant difference ($p<0.05$) between *Callinectes pallidus* caught in February and Junewhile moisture content, crude protein and crude fibreshowed no significant difference ($p<0.05$). The percentage eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for *C. pallidus* caught in June (18.47%, 10.53%) was higher than those caught in February (11.21%, 6.90%) while the n-6/n-3 ratio of *C. pallidus* caught in February (1.07) was higher than those caught in June (0.71). Glutamic acid had the highest concentration of non-essential amino acid in *C. pallidus* caught in

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both seasons while leucine had the highest concentration of essential amino acids. This study showed that *C. pallidus* caught in wet season had higher crude protein content, polyunsaturated fatty acids and essential amino acids than *C. pallidus* caught in dry season.

Keywords: Season; dry; wet; February; June; eicosapentaenoic; docosahexaenoic; Callinectes pallidus.

1. INTRODUCTION

Crabs which are the basic components of the ecosystem belong to the group of shellfish called crustaceans and the phylum Arthropoda [1,2]. Crabs are widely found in tropical areas, they do not only serves as food but are also used as fertilizers. It has been reported that crustaceans account for about one-fifth of all the foods obtained from all aquatic sources [3] with crab making up to about the same one-fifth of all marine crustaceans consumed worldwide [4]. Fish is usually rated higher in preference to crabs which are considered inferior and food for the low income earners due to its low price.Crab is used in a wide variety of dishes due to its high market availability, and various health benefits such as a rich source of high quality protein, minerals and vitamins [5,6,7]. It was reported that the protein in crustaceansis high in essential amino acids and is highly digestible due to the lack of connective tissue [1].

Callinectes pallidus (The Gladiator swimming crab) is an economically important species commonly soldin the open markets throughout the coastal towns ofLagos, Nigeria [8]. This crab species feeds on fish, molluscs, crustaceans, macroscopic plants, algae and sand particles [9,10] with males consume more fish while females consume more plants. Just like fish, the chemical composition of crabs varies greatly among species and from an individual crab to another, depending on age, sex, environment, and season [11]. This research work is aimed at investigating the effect of seasonal fluctuations on the nutrient content of the whole body of *Callinectes pallidus*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

20 individuals each of *Callinectes pallidus* (The Gladiator swimming crab) were captured during dry (February, 2019) and wet seasons(June, 2019) and collected from the landing site of Lekki Lagoon, Lagos State, Nigeria. The crab samples were transferred to the laboratory using ice box and washed properly. The whole crab samples were dried in the oven and grinded. The grinded samples werestored in air tight containers and kept in the freezer for further analysis.

2.2 Description of Sample Area

Lekki lagoon which lies between longitudes 4º 00' and 4º 15' E and between latitudes 6º 25' and 6º 37' N is located in Lagos State, Nigeria [12,13]. It has a surface area of about 247 km² with a maximum depth of 6.4 m at low tide and mean depth of 3.1 m [14,15,16]. The lagoon which is surrounded with many beaches is being fed by River Oni discharging to the North-eastern and Rivers Oshun and Saga discharging into North-western parts [12,13]. This lagoon is the most important source of freshwater fish in Lagos State [12]. Lekki lagoon experiences both dry and rainy seasons which is common to southern part of Nigeria [13].

2.3 Determination of Proximate Components of the Crab Samples

The crude fat, crude fibre, ash content and carbohydrate content of the crab samples were determined according to the methods of [17] while the crude protein was determined according to the method of [18] with some modifications.

2.3.1 Moisture content

The samples (5g) each was dried in an oven at 105ºC for 6 hours, it was cooled in the desiccator and weighed again. This process was repeated until constant weight was obtained. The percentage moisture content was calculated as:

$$\% \text{ Moisture} = \frac{(Weightlossduetodrying)X\ 100}{(Weightofsample)}$$

2.3.2 Crude fat

The samples (5 g) each were weighed into a thimble and placed in a Soxhlet apparatus. A 500 ml round bottom flask was attached to the base of the extractor and clamped to a retort stand.

300 mL petroleum ether was poured into the thimble. The set up was placed on heating mantle with the top of the extractor connected to the reflux condenser. The source of heat was turned on as well as water source supplied to enable the solvent in the flask to boil and extract the lipid in the sample. The extraction was completed in 12 hours and the solvent was recovered using rotary evaporator. The extracted lipid in the flask was placed in an oven at 70 °C for 30 mins to completely remove all the solvent residues and then placed in a desiccator to cool. The percentage of lipid was calculated using the equation below:

Weight of lipid = Weight of flask and content after extraction – Weight of flask before extraction

2.3.3 Crude protein

The crude protein content was determined using microkjeldahl method. The digested samples were diluted, made alkaline with NaOH and distilled water. Liberated ammonia gas was trapped in a conical flask containing boric acid solution. The conical flask was positioned such that the stem of the condenser dipped into the boric acid solution. After collecting about 50cm³of the distillate, the receiver was lowered and the tip of the condenser was washed with distilled water, the ammonia solution in the distillate was titrated against 0.1M HCl. A blank determination was carried out using the same amount of the reagents in the absence of the sample.

$$\% \text{ Nitrogen Content}=\frac{(TitrevalueXMX\ 0.0014\ XDfXCf)}{(Weightofsample)}$$

Where:
M = Molarity of HCl = 0.01M
Df = Dilution factor = 50
Cf = Correction factor = 10
% Crude protein = % Nitrogen x 6.25
% Nitrogen was converted to percent crude protein by multiplying with 6.25, the conversion factor. Most proteins contain 16% Nitrogen, hence, the conversion factor is 6.25 (100/16 = 6.25).

2.3.4 Crude fibre

100 ml of 0.25M H_2SO_4 was added to 2g each of the samples and brought to boil for 30 mins after which the hot mixture was filtered. The residue was washed free of acid with plenty of warm water. Each residue was then transferred into round bottom flasks to which 100ml of 0.25M of NaOH was added and boiled again for 30minutes. The mixture was then filtered and the residue washed free of alkali with warm water. The residue was then transferred to a dried, weighed silica dish and dried to a constant weight at 105°C for 90 mins, cooled in a desiccator and weighed. The weighed samples were burnt off and reweighed. The percentage crude fibre content was determined as follows:

Initial weight of residue - Final weight of residue x 100

2.3.5 Ash content

The sample (5g) each was weighed into a previously dried, cooled and weighed silica crucible. The crucible containing the sample was transferred into a muffle furnace and ignited at 550°C until a white ash was obtained. The ash was moistened with distilled water, dried on steam bath and then on hot–plate and re-ashed at 550ºC to constant weight. The percentage ash content was calculated as follows:

$$\text{Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample}}\ \text{X}\ 100$$

2.3.6 Carbohydrate

The carbohydrate content was determined by difference i.e.
% Carbohydrates= 100 - (%Mo + % As + % Cf + % Cp)
Where; %Mo= Percentage moisture content
%As= Percentage ash content
%Cf= Percentage crude fat
%Cp= Percentage crude protein

2.4 Determination of Fatty Acids Composition of the Crab Samples

The lipids gotten from the crude fat determination was evaluated for the fatty acid compositions according to the method of [19] with some modifications. Fatty acids were transesterified to methyl esters with 0.5 M KOH in methanol solution. 50 µL each of oil samples was placed into 10 mL centrifugal tubes to which 5 mL of the prepared KOH–MeOH solution was added. The fatty acid methyl esters were recovered with hexane. For the identification of the methyl esters of fatty acids a Hewlett Packard HP 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 mass spectroscopy detector (GC-MS) system was used equipped with a methyl lignoserate-coated stationary phase, capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) and a flame ionization detector. The temperature program was set up from 50°C to 250ºC with 4ºC/min, both the injector and detector temperatures were 280°C respectively.

The oven temperature was programmed for 3.5 min at an initial temperature of 150C, was increased at a rate of 20C/min to 200C, was further increased at a rate of 5C/min to 280C, and then held at that temperature for 6 min. Helium was used as a carrier gas at a pressure of 290 kPa, with a flow velocity of 1 mL/min. The values of fatty acids are presented as area percentage of total fatty acids.

2.5 Determination of Amino Acid Composition of the Crab Samples

The amino acid profile of the samples were determined using amino acid analyser, technicon TSM-1 (model: DNA 0209) and methods described by [20]. The crabsamples were dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-sample Amino Acid Analyser (TSM). Approximately 200 mg each of the fish samples was dissolved in 0.7 mL distilled H_2O and0.5 mL 20 mM norleucine (internal standard). 500mL each of the extract was mixed with 50 mL 20 mM norleucine. Concentrated hydrochloric acid (HCl, 12 M) was added, to a final concentration of 6 M. The sample mixture was flushed with nitrogen gas for 15 s in order to minimize oxidation, before hydrolysis at 110°C for 24 h. Following hydrolysis, 100 mL aliquots of the hydrolysates were evaporated under nitrogen gas until complete dryness and re-dissolved to a suitable concentration in lithium citrate buffer at pH 2.2. All amino acids were analysed chromatographically using an ion exchange column followed by ninhydrin post column derivatization on a Biochrom 30 amino acid analyser (Biochrom Co., Cambridge, UK).

2.6 Statistical Analysis

Statistical analyses were performed using SPSS (version 20). All data collected were subjected to independent sample T-test analysis. Means were used to compare differences between the treatment means at 5% probability level.The means were separated using LSD at 0.5%.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of the Crab Samples

Table1shows the proximate composition of the crab samples (*C. pallidus*) caught in February and June periods. Parameters such as crude fat(2.27%±0.015^a for Februaryand 0.73%±0.034^b for June), ash content (5.01%±0.026^{a}for Februaryand 3.97%±0.017^b for June)and carbohydrate (2.87%±0.04^a for Februaryand 2.14%±0.019^b for June) of the *C. pallidus* showed significant difference ($p<0.05$) between February and Juneperiods. However, moisture content, crude protein and crude fibreshowed no significant difference ($p<0.05$) between these two periods. The highest values for crude fat, ash content and carbohydrate contents were found in *C. pallidus* caught in February.

The proximate composition of the crabs showed high moisture (73.45%), crude protein (18.45%) and crude fibre (1.26%) contents in crabs caught in June. This result was related to (77.11%-78.50%) and (19.18%-20.21%) reported by [21] for *C. sapidus* collected between the months of February-December for moisture and crude protein contents respectively. The moisture content obtained in this study was higher than (53.56%) reported by [22] for *C. pallidus* captured from Badagry creek. Thiscould be due to difference in sizes, age or habitat. High moisture content in crab helps in stabilization of the crab during movements [21]. The high protein content showed that crab meat can supply sufficient protein in diet and protein is among the classes of food which is essential for growth and body defence [21,23].

The ash contents (3.97% and 5.01%) obtained in this study was lower than 13.41% reported by [22] for *C. pallidus* but in close range with 2.18%

Table 1. Proximate composition of the crab samples

Parameters (%)	*C. pallidus* (February)	*C. pallidus* (June)
Moisture content	69.62±0.05	73.45±0.023
Crude protein	18.11±0.06	18.45±0.018
Crude fat	2.27±0.015^a	0.73±0.034^b
Crude fibre	1.12±0.004	1.26±0.023
Ash content	5.01±0.026^a	3.97±0.017^b
Carbohydrate	2.87±0.04^a	2.14±0.019^b

Values are mean ± standard deviation of triplicate determinations

^{ab}: Means within each row with different superscripts are significantly different ($p<0.05$)

reported by [21] for *C. sapidus* collected between the months of February-December. The ash content of crabs collected during dry season was higher than those collected during rainy season, it may be as a result of *C. pallidus* captured during dry season having more bony tissue than those collected during rainy season [4]. The fat content was higher in dry season (2.27%) than in rainy season (0.73%).

Comparing the proximate composition in the two seasons, *C. pallidus* captured in February possess higher moisture, ash and crude fat contents than those captured in June. These differences could be related to environmental variations, like changes in temperature or in nutrients availability, as well as differences in animal physiology [24].

3.2 Fatty Acids Composition of the Crab Samples

Table 2 shows the fatty acids composition of the crab samples (*C. pallidus*) in February and Juneperiods. For SFA, the significant ones were myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0) and behenic acid (C22:0). However, the insignificant SFA include lauric acid (C12:0), magaric acid (C17:0) and lignoceric acid (C24:0). For MUFA: the significant fatty acids were oleic acid (C18:1n-9c), vaccenic acid (C18:1n-7c), C18:1n-9t and erucic acid (C22:1n-13c). The insignificant ones among MUFA include Myristoleic acid (C14:1), palmitoleic acid (C16:1), C18:1n-6c, cetoleic acid (C22:1n-11c), C22:1n-9 and nervonic acid (C24:1n-9). For PUFA, the significant fatty acids include linoleic acid (C18:2n-6c), α-linolenic acid (C18:3n-3), $_{\gamma}$-linolenic acid (C18:3n-3), eicosadienoic acid (C20:2n-6), arachidionic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3), docosadienoic acid (C22:2n-6) and docosahexaenoic acid (DHA) (C22:6n-3). However, the insignificant ones include C18:2n-6t, rumenic acid (C18:2n-7) and dihomo-gamma-linolenic acid (DGLA) (C20:3n-6).

This fatty acid composition revealed that saturated fatty acids were more abundant in *C. pallidus* caught during dry season. Palmitic and stearic acids were the abundant saturated fatty acids in the crabs caught in both seasons. This result was in agreement with the report of [25] that also reported palmitic and stearic acids as the abundant saturated fatty acid. Oleic acid had the highest concentration in all the monounsaturated fatty acids in the profile. This profile was closely related to those reported by [25,26] that also reported oleic acid as the monounsaturated fatty acid with highest concentration.

The percentage EPA for *C. pallidus* caught in June (18.47%) was higher than those caught in February (11.21%).EPA and DHA play important roles in the prevention of inflammatory and cardiovascular diseases due to their serum triglycerides-lowering effects [27]. The n-6/n-3 ratio was 1.07 and 0.71 for *C. pallidus* caught in February and June respectively.The adequate n-6 / n-3 ratio (less than 4) is critical to formation of eicosanoids, preventing the development of various diseases [28]. The type and quantity of essential fatty acid consumed and balanced intake of omega-3 and omega-6 are important for a healthy life. Food rich in omega-3 fatty acids should be made abundant in consumption than food rich in omega-6 fatty acids in order to prevent chronic disease [29]. The PUFA/SFA ratio was 0.78 and 1.58 for *C. pallidus* caught in February and June respectively. A diet with low PUFA / SFA ratio is not recommended as it is a risk factor for the increase in serum cholesterol [30]. The fatty acid composition showed that the concentration of saturated fatty acids increased in *C. pallidus* caught in February while the concentration of the polyunsaturated fatty acids reduced. This could be as a result of adaptation to temperature as the accumulation of long-chain PUFA before the cold season could make crabs more able to adapt to rapid decreases in temperature and the accumulation of saturated fatty acids before the hot season more capable of adapting to rapid increases in temperature [26,31]. Fatty acids may play an important role in the adjustments of membrane fluidity to variations in environmental temperature [26].

3.3 Amino Acids Composition of the Crab Samples

Table3 shows the amino acid composition of the crab samples (*C. pallidus*) in February and June periods. For EAA, the significant ones were histidine, lysine, methionine and tryptophan. However, the insignificant EAA includeisoleucine, leucine, phenylalanine, threonine valine.For NEAA: the significant ones

Table 2. Fatty Acid composition of the crab samples

Fatty Acids (%)	*C. pallidus* (February)	*C. pallidus* (June)
Lauric acid (C12:0)	0.02±0.041	0.01±0.022
Myristic acid (C14:0)	1.20±0.016[a]	0.42±0.034[b]
Palmitic acid (C16:0)	25.11±0.06[a]	17.77±0.03[b]
Magaric acid (C17::0)	0.01±0.045	0.01±0.033
Stearic acid (C18:0)	15.04±0.05[a]	10.42±0.024[b]
Arachidic acid (C20:0)	1.53±0.033[a]	0.92±0.018[b]
Behenic acid (C22:0)	1.35±0.032[a]	0.44±0.041[b]
Lignoceric acid (C24:0)	0.02±0.024	0.01±0.03
∑SFA	44.28	30.00
Myristoleic acid (C14:1)	ND	0.02±0.026
Palmitoleic acid (C16:1)	0.05±0.015	0.14±0.043
C18:1n-6c	3.97±0.027	4.22±0.029
Oleic acid (C18:1n-9c)	15.69±0.06[b]	17.11±0.03[a]
Vaccenic acid (C18:1n-7c)	0.05±0.039[a]	0.04±0.028[b]
C18:1n-9t	0.12±0.018[a]	0.09±0.04[b]
Cetoleic acid (C22:1n-11c)	0.01±0.052	0.06±0.031
Erucic acid (C22:1n-13c)	1.13±0.045[a]	1.02±0.033[b]
C22:1n-9	0.01±0.025	0.03±0.044
Nervonic acid (C24:1n-9)	0.03±0.038	0.02±0.018
∑MUFA	21.06	22.75
C18:2n-6t	0.02±0.04	0.01±0.032
Rumenic acid (C18:2n-7)	0.01±0.026	0.01±0.018
Linoleic acid (C18:2n-6c)	6.23±0.04[b]	7.14±0.025[a]
α-Linolenic acid (C18:3n-3)	0.56±0.043[b]	1.02±0.02[a]
γ-Linolenic acid (C18:3n-6)	0.50±0.025[b]	0.77±0.033[a]
Eicosadienoic acid (C20:2n-6)	1.99±0.046[b]	2.33±0.05[a]
Dihomo-gamma-Linolenic acid (DGLA) (C20:3n-6)	0.16±0.027	0.15±0.024
Arachidionic acid (C20:4n-6)	6.97±0.044[a]	6.67±0.031[b]
Eicosapentaenoic acid (C20:5n-3)	11.21±0.025[b]	18.47±0.03[a]
Docosadienoic acid (C22:2n-6)	0.11±0.023[b]	0.15±0.03[a]
Docosahexaenoic acid (DHA) (C22:6n-3)	6.90±0.032[b]	10.53±0.018[a]
∑PUFA	34.66	47.25
n-6	19.95	21.44
n-3	18.67	30.02
n-6/n-3	1.07	0.71
% EPA	11.21	18.47
% DHA	6.90	10.53
PUFA/SFA	0.78	1.58

Values are mean ± standard deviation of triplicate determinations

[ab]*: Means within each row with different superscripts are significantly different (p<0.05)*

were alanine, aspartic acid, glycine and tryosine. The insignificant NEAA include arginine, cystine, glutamic acid, proline and serine.

The amino acid composition of the *C. pallidus* caught in both seasons revealed they both contain high amount of non-essential amino acids. Glutamic acid had the highest concentration of non-essential amino acid in *C. pallidus* caught in both seasons while leucine had the highest concentration of essential amino acids. This result is in agreement with those reported by [25] and [26] for *C. amnicola* and *C. maritae*. Some non-essential amino acids such as cysteine, tyrosine, glycine, arginine, glutamine, or proline, are termed conditionally indispensable since they became essential under specific pathological or physiological conditions [32]. It was observed that there was no significant difference in the amino acid profile of *C. pallidus* caught in February and June.

Table 3. Amino Acid composition of the crab samples

Amino Acids (g/100g)	*C. pallidus* (February)	*C. pallius* (June)
Histidine	1.88±0.026[b]	2.01±0.037[a]
Isoleucine	4.11±0.05	3.66±0.028
Leucine	11.76±0.033	12.38±0.042
Lysine	11.54±0.06[a]	11.16±0.019[b]
Methionine	4.41±0.035[a]	3.99±0.044[b]
Phenylalanine	4.70±0.016	3.60±0.03
Threonine	4.15±0.054	3.97±0.022
Tryptophan	1.74±0.04[b]	1.83±0.028[a]
Valine	3.90±0.023	3.65±0.015
∑EAA	48.19	46.25
Alanine	3.05±0.023[b]	4.11±0.05[a]
Arginine	9.14±0.025	8.95±0.018
Aspartic acid	13.42±0.05[a]	13.23±0.026[b]
Cystine	1.39±0.036	1.36±0.024
Glutamic acid	14.26±0.042	14.15±0.031
Glycine	1.98±0.022[a]	1.95±±0.019[b]
Proline	3.56±0.036	3.47±0.028
Serine	3.11±0.033	3.15±0.024
Tryosine	1.87±0.03[a]	1.73±0.014[b]
∑NEAA	51.78	52.10
TAA	99.97	98.35

Values are mean ± standard deviation of triplicate determinations

[ab]*: Means within each row with different superscripts are significantly different (p<0.05)*

4. CONCLUSION

The results obtained in this study showed that *C. pallidus* caught during wet season had higher crude protein, polyunsaturated fatty acids and essential amino acids than *C. pallidus* caught during dry season. Also, this study suggests whole *C. pallidus* as food that could give healthy living and balanced human nutrition regardless of the season being caught.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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