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EFFECT OF SIZE ON PROXIMATE COMPOSITION AND HEAVY METAL CONTENT OF THE MANGROVE OYSTER *CRASSOSTREA GASAR* FROM THE ANDONI RIVER, NIGERIA

Woke, G. N.[†], Umesi, N.[* †], & Oguzor, N. S.[‡]
[†]*Department of Animal & Environmental Biology, University of Port Harcourt, Nigeria.*
[‡]*Department of Agronomy, Michael Okpara University of Agriculture, Umudike, Nigeria.*

ABSTRACT: *The proximate composition and heavy metal content of the mangrove oyster Crassostrea gasar from the Andoni River was investigated. Samples were categorized into three class sizes: small (< 25 mm), medium (25-50 mm), and large (> 50 mm) each with 10 sampling units for each class size. Differences across class size were significant for carbohydrate, lipid, and fiber content with their highest mean values recorded as 58.39 ± 0.21%, 6.70 ± 0.10% and 18.22 ± 0.28% respectively. Size-specific differences were detected only for concentrations of lead and zinc which showed maximum concentrations of 0.53 ± 0.02μg/g and 42.69 ± 1.81μg/g respectively. The proximate composition and heavy metal content recorded were generally higher in large-sized oysters and the variations observed were attributed to obvious differences in their class sizes. The remarkably low moisture content recorded in C. gasar was attributed to higher drying temperatures (105°C). Food quality and uptake of heavy metals in tissues of C. gasar generally varied along size gradients, with peak values in large-sized individuals. However, the levels of heavy metals recorded in soft tissues of C. gasar in this study were generally very low and fall within levels expected in tissues of aquatic biota.*

KEYWORDS: Proximate, Heavy Metal, Content, Oyster & Size

INTRODUCTION

Fish and fishery products constitute major and cheap sources of animal protein. Despite this, they also account for only 40% of the diet of an average Nigerian. Nigeria has vast potentials for fisheries development, being endowed with a maritime area of 46,300 km^2, an Exclusive Economic Zone (EEZ) area of 210,900 km^2 and inland waters of 12.5 million ha. However, in spite of the huge endowment, the current production level of 400,000 metric tons in the country is at a 50% deficit to meet Nigeria's fish need per annum of at least 1.5 million metric tons. Consequently, the need to supplement current production from artisanal fishing industry has necessitated Nigeria to import about 49.5% of fish (Allison and Okadi, 2009).

Small scale artisanal and commercial fisheries harvest oysters of the genus Crassostrea for food, sales, and shells which provide ingredients for animal food industries (FAO,

[*] Correspondence: ndbc_umesi@yahoo.com

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1982; Abiogba and Henadou, 2006; Adite *et al*., 2013). The Crassostrea genus has widespread occurrence across the globe, and biologists have taxonomically identified and classified over 54 species of oysters into this genus (Miossec *et al*., 2009; Abgrall *et al*., 2010) including *Crassostrea gigas* (Japanese oyster), *Crassostrea angulata* (Portuguese oyster), *Crassostrea virginica* (Eastern oyster), *Crassostrea rhizophorae* (Brazilian oyster), and *Crassostrea gasar* (West African oyster). Among the Crassostrea, C. gigas ranks amongst the most cultivated species. According to Adite *et al*., (2013), wild collections and cultivation of oysters of the Crassostrea genus, particularly *C. gasar*, provides immense economic benefits to riparian communities.

Oysters are, therefore, commercially important and are exploited mainly for their flesh. In Southern Nigeria, the Andoni River represents one of the main fishing grounds of oysters which represent a viable source of income and livelihood not only for the Andoni and Khana communities, but also for fish mongers in neighbouring communities and Port Harcourt who buy this seafood in bulk from the Kaa waterside market in Khana Local Government Area of Rivers State, Nigeria. Oysters are relatively more sedentary and are filter feeders with greater chances and capacities of accumulating heavy metals from sediments in their tissues (El-Shenawy, 2002) to levels that may pose human health risks (Gunther *et al*., 1999; Nasci *et al*., 1999; Olivier *et al*., 2002). Given that most oysters harvested in the Niger Delta area ultimately ends up in our tables as delicacies, it is very important to monitor periodically their food values and levels of various heavy metals of environmental and human health concern in their tissues. The objective of this study is to determine the effect of size on the proximate composition and heavy metal content of the mangrove oyster *Crassostrea gasar* caught by fishermen from the Andoni River in Rivers State, Nigeria.

MATERIALS AND METHODS

Study Area

The study area is a narrow stretch of the Andoni River, Rivers State, Nigeria, adjacent to the fish market located at Kaa Waterside in Khana Local Government Area of Rivers State. The study area which lies in latitude 4° 34.18′N and longitude 7° 22.10′E represents an important landing site of a wide variety of fin- and shell-fishes caught by local fishermen from the Andoni River.

Study Design

The elemental composition of animal materials varies greatly and is governed by several abiotic factors. In this study, different class sizes of of the oyster *Crassostrea gasar* from the Andoni River, Rivers State, were analysed for proximate composition (ash, moisture, carbohydrate, protein, lipid, and fiber content) and heavy metal content (cadmium, chromium, copper, mercury, lead, zinc, and arsenic.

Laboratory Analyses

In the laboratory, samples of *C. gasar* were first cleansed to remove mud and any debris and then washed with distilled water. Samples were then categorized into three class sizes

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each with 10 sampling units for the small (<25 *mm*), medium (25-50 *mm*), and large (> 50 *mm*) class size categories, based on shell length (*mm*). Soft tissues of *C. gasar* were extracted from their shells using a sterile stainless steel knife as recommended in previous studies (Chiu *et al*., 2000; Koh *et al*., 2011; Madkour *et al*., 2011). Soft tissues of *C. gasar* were dried by thinly spreading them in a hot-box oven at a temperature of 105ºC for 48 h (Fashina-Bombata and Megbowon, 2012), until they were sufficiently dry to be ground. Porcelain mortar and pestle were used to grind sampling units of each class size to fine powdery form and then screened through a sieve of 2 *mm* mesh size (Murtala *et al*., 2012) for analyses of proximate composition and heavy metal content.

Proximate Composition

Proximate analysis was carried out on two (2) replicates according to procedures described by Allen *et al.* (1974) in Chemical Analysis of Ecological Materials. Ash content was determined by ashing samples in an electric muffle furnace at 500°C for 3 h. Moisture content was determined by heating 1g oven-dried finely ground sample in a hot box oven at 105°C to a constant dry weight. Protein content was determined by the Kjeldahl method in which the total nitrogen content of the sample was first estimated from which crude protein content was estimated by multiplying by the conventional factor of 6.25 (i.e. %N x 6.25). Lipid was estimated by the batch solvent extraction technique in which lipid was extracted using diethyl ether as solvent and the lipid content of sample determined by evaporating the solvent (Fashina-Bombata and Megbowon, 2012). The carbohydrate content was estimated as the Nitrogen-Free-Extract (NFE) by subtracting the sum of the weights of protein, fiber, and ash from the total dry matter of sample.

Heavy Metals

Acid digestion of samples was carried out according to procedures described in Allen *et al*. (1974). Approximately 1g oven-dried, ground and sieved sample was weighed into a 100ml kjeldahl flask to which was added 1ml 60% perchloric acid ($HCIO_4$), 5ml nitric acid (HNO_3) and 0.5ml sulphuric acid (H_2SO_4). The flask was swirled gently and then digested slowly at moderate heat, increasing later. Sample was digested for 10-15 minutes and was set aside to cool after the appearance of white fumes. Sample was then filtered (No. 44 paper) into 50ml volumetric flask and diluted to volume, and used for the determinations of the heavy metals cadmium, chromium, copper, mercury, lead, zinc, and arsenic using the atomic absorption spectrophotometer.

Data Analysis

Data generated from the study were analysed using the Data Analysis Toolpak of the Microsoft Excel. Bar charts were used to depict trends in chemical profiles of the animals used in the study. Analysis of Variance (ANOVA) was used to test for significant differences in means in proximate composition as well as heavy metal content of the animals at the 95% significance level. Where differences in ANOVA were significant, the Tukey Multiple Range Test was used as basis for distinguishing mean differences which were significantly different (Fowler *et al*., 1998).

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RESULTS

Slight variations which were not found to be significant were observed in the mean ash, moisture, and protein contents of soft tissues of the mangrove oyster *Crassostrea gasar* across the three class sizes: small-sized (<25 *mm*), medium-sized (25-50 *mm*), and large-sized (> 50 *mm*). Mean ash (Fig. 1(a)) and moisture (Fig. 1(b)) contents tended to be higher in the medium-sized oysters compared to the small-sized and large-sized oysters. Protein content, on the other hand, recorded a slightly higher mean value in the large-sized oyster samples (Fig. 1(d)) than means of the other two class sizes. These observed variations were, however, not significant ($p > 0.05$, Table 1).

Result of one-way analysis of variance (ANOVA) evaluating the effect of class size on carbohydrate content of *C. gasar* was significant ($F_{2,3} = 322.929$, $p < 0.05$, Table 1). Mean carbohydrate content was higher in the large-sized oysters (> 50 *mm*), compared to the small-sized (< 25 *mm*) and medium-sized (25-50 *mm*) individuals (Fig. 1(c)). Evidence was obtained from sample data that carbohydrate content of soft tissues of *C. gasar* was influenced by the size of the animal as large-sized *C. gasar* showed a significantly higher mean value compared to the small-sized and medium-sized samples. A significant difference was also detected in mean carbohydrate content between tissue samples of small-sized and medium-sized *C. gasar*, with a higher mean value recorded in the later (Tukey, $p < 0.05$).

The ANOVA on lipid content of soft tissues of *C. gasar* was also found to be significant ($F_{2,3} = 14.948$, $p < 0.05$, Table 1). Slight variability in lipid content was observed in tissues of small-sized (< 25 *mm*), medium-sized (25-50 *mm*), and large-sized (> 50 *mm*) samples of the animal, with the lowest and highest mean values recorded in the small-sized and large-sized oysters respectively (Fig. 1(e)). Among *C. gasar*, larger individuals appear to accumulate higher lipid content in tissues relative to individuals which are smaller in size. Although no significant difference was detected in lipid content between the medium-sized and large-sized oysters, as well as the small-sized and medium-sized oysters, the large-sized individuals showed a significantly higher mean value compared to the small-sized individuals (Tukey, $p < 0.05$).

The result of ANOVA on mean fiber content of C. gasar showed differences which were significant between class size ($F_{2,3} = 25.985$, $p < 0.05$, Table 1). Mean fiber content of *C. gasar* showed roughly similar trend as carbohydrate content, with mean values increasing from a minimum in small-sized (< 25 *mm*) to a maximum in large-sized (> 50 *mm*) oysters. Mean fiber values obtained in small-sized and medium-sized oysters were fairly similar and lower than the peak value recorded in the large-sized individuals (Fig. 1(f)). Size tended to show a direct relationship with fiber content of C. gasar as mean value of fiber in tissues of the animal was higher in the large-sized compared to small-sized individuals. Differences in mean fiber content between the small-sized and medium-sized oysters were not found to be significant. However, a significantly higher mean value was recorded in samples of large-sized oysters compared to the small-sized and medium-sized oysters (Tukey, $p < 0.05$).

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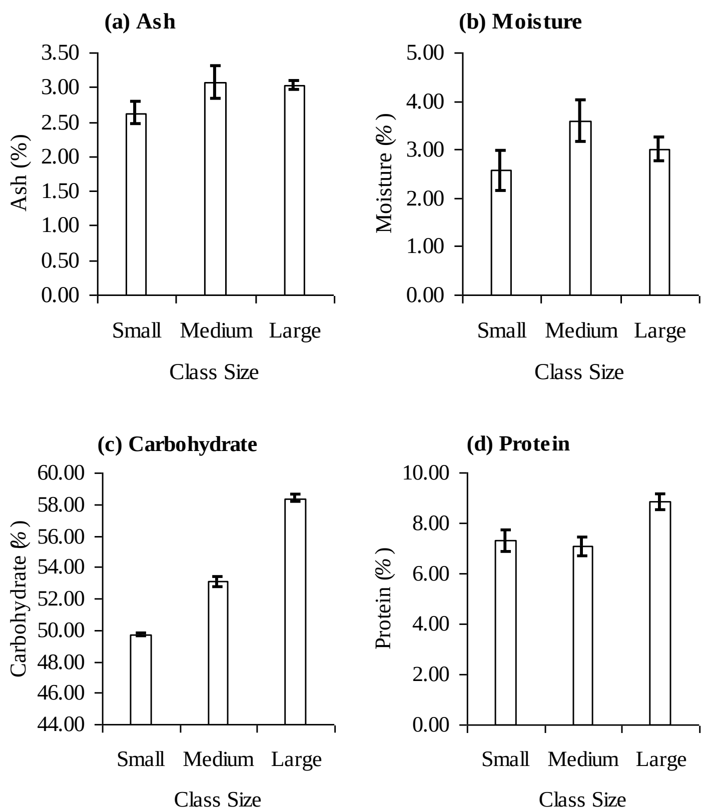
Although the lowest and highest mean values of copper (Cu) in soft tissues of *C. gasar* were recorded in the small-sized (< 25 *mm*) and large-sized (> 50 *mm*) samples respectively (Fig. 2(b)), the observed differences in means across the three class sizes of the animal were not significant ($p > 0.05$, Table 2). The ANOVA on lead (Pb) content of tissues of *C. gasar* was found to be significant ($F_{2,3} = 117.444$, $p < 0.05$, Table 2). The small-sized (25 *mm*) and medium-sized (25-50 *mm*) oysters showed mean Pb values that were fairly similar, while a comparatively higher mean value was obtained in the large-sized (> 50 *mm*) oysters (Fig. 2(c)). The concentration of Pb in soft tissues of *C. gasar* seemed to also vary with size given that the mean value of Pb recorded in the large-sized oyster samples was relatively higher than those of the other two class sizes. No significant difference was found in Pb content of the small-sized and medium-sized oysters. On the other hand, large-sized oysters showed a significantly higher mean Pb value compared to the small-sized and medium-sized samples (Tukey, $p < 0.05$).



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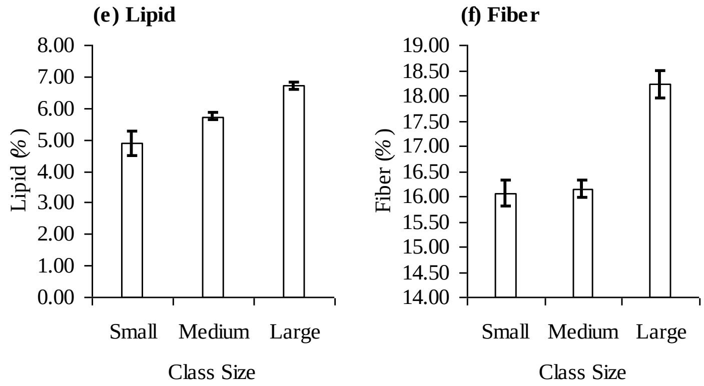
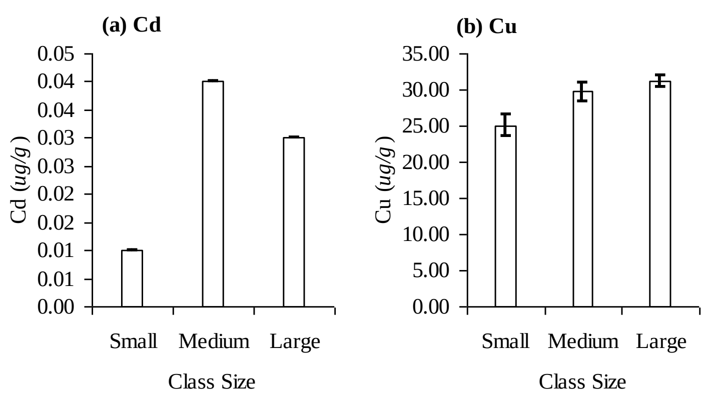


Fig. 1 Proximate composition (mean ± SE, n = 2) of tissues of 3 class sizes of *Crassostrea gasar* from the Andoni River.

Table 1 One-way analysis of variance on proximate composition (%) of *Crassostrea gasar* from the Andoni River.

Composition	df	MS	F	P-value
Ash	2	0.121	2.172	> 0.05
Moisture	2	0.529	1.940	> 0.05
Carbohydrate	2	38.606	322.929	< 0.05*
Protein	2	1.851	6.901	> 0.05
Lipid	2	1.687	14.948	< 0.05*
Fiber	2	3.020	25.985	< 0.05*

* Significant at $p < 0.05$



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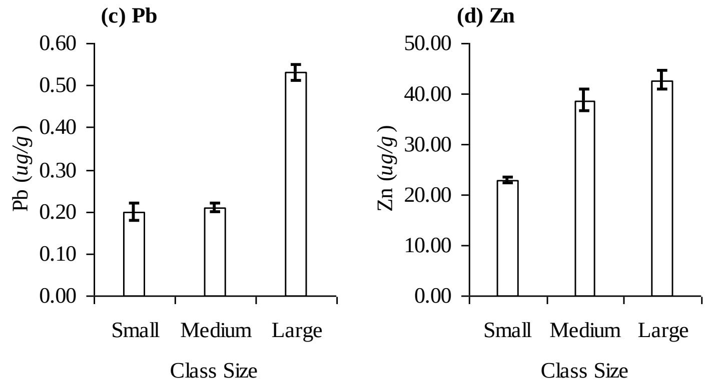

Fig. 2 Concentrations (mean ± SE, n = 2) of heavy metals (*µg/g*) in tissues of 3 class sizes of *Crassostrea gasar* from the Andoni River.

Table 2 One-way analysis of variance on heavy metal content (*µg/g*) of *Crassostrea gasar* from the Andoni River.

Metal	df	MS	F	P-value
Copper	2	21.045	6.879	> 0.05
Lead	2	0.070	117.444	< 0.05*
Zinc	2	219.147	39.439	< 0.05*

* Significant at $p < 0.05$

The ANOVA on mean zinc (Zn) content of soft tissues of C. gasar was significant ($F_{2,3} = 39.439$, $p < 0.05$, Table 2). Mean values of Zn recorded in *C. gasar* also ranged from a minimum in small-sized animals (< 25 *mm*) to a maximum in large-sized animals (> 50 *mm*). Mean Zn values observed in the medium-sized and large-sized oysters were identical and both higher than the minimum value obtained in the small-sized oysters (Fig. 2(d)). No significant difference was found in mean Zn content of the medium-sized and large-sized oysters. However, the small-sized oysters recorded a mean value that was found to be significantly lower than those obtained in the medium-sized and large-sized samples.

DISCUSSION

After proteins and lipids, carbohydrates represent the third most abundant group of organic compounds in the animal's body. Apart from providing an important source of energy, digestible carbohydrates also contribute greatly to the quality (sweetness, appearance, and textural characteristics) of many foods. The mean carbohydrate content of *Crassostrea gasar* recorded in this study varied 49.68 ± 0.07 – 58.39 ± 0.21% (Fig. 1(c)). This range was higher than that (4.21 – 15.67%) reported by Swapna and Ravinder (2015) in samples of the bivalve *Parreysia cylindrical* and mean values recorded by Thivakaram (1988) in *Nodilittorina. pyramidalis* (4.69%) and *Littorina. quadricentus* (5.31%).

Lipids also represent a major source of energy and provide essential lipid nutrients that feature in our diets. Like carbohydrates, lipids also have been documented to play a key role in determining the overall physical attributes (flavour, texture, mouthfeel, and appearance) of foods. The range of values of lipid obtained in *C. gasar* in this study agrees with that (5.34 – 6.63%) reported as high lipid content by Swapna and Ravinder (2015) in *Parreysia cylindrical*. The results of this study also agrees with Omotoso (2005) who reported lipid content that fell in the interval 5.35 ± 0.01% in dried *Callinectes armatum*.

Liberal consumption of fiber plays an important role in preventing or at least reducing the incidence of colon cancer, cardiovascular disease, and constipation. The results of this study recorded high fiber content in soft tissues of *C. gasar* (16.05 ± 0.25 – 18.22 ± 0.28%) (Fig. 1(f)). Values recorded in oysters in this study were found to greatly exceed the range of values (6.690 – 11.070%) reported by Moronkola *et al*. (2011).Lead concentrations reported in different species of infaunal bivalve (*Macoma balthica*, *Macoma nasuta*, *Chione subrugosa*, *Cerastoderma edule*) around the world are normally between 1 and 4 *μg/g dw*, reaching values of 8 *μg/g* in some moderately polluted sites (Szefer *et al*., 1998; Sokolowski *et al*., 2002, 2007; Lu *et al*., 2005; Jung *et al*., 2006). Mean values of lead recorded in this study were very low and ranged from 0.20 ± 0.02 – 0.53 ± 0.02*μg/g* (Fig. 2(c)). Similar studies conducted on oysters from lagoon systems in the Southern Gulf of Mexico recorded a mean value of 189.78*μg/g* which was several orders of magnitude higher than the range of values of lead obtained in *C. gasar* in the present study. The results of the present study also differed from lead concentrations of 1.5 – 8.5*μg/g* reported in *Dosinia exoleta* (individuals of shell length <40*mm*) from shellfish extraction areas in the Galician Rias (NW Spain). However, concentrations up to 20 *μg/g* were also reported in individuals of the species of shell length 45 *mm* (Sanchez-Marina and Beiras, 2008).

Wardlaw and Smith (2009) have catalogued health benefits associated with Zn in diets including the ability to heal wounds, develop sexual organs and bones, improve the release and function of insulin, and enhance immune function. The values of Zn obtained in this study fell in the range 28.89 ± 0.53 – 42.69 ± 1.81 *μg/g*. In a study conducted on *C. amnicola*, Moronkola *et al*. (2011) reported Zn levels (128 – 388 *μg/g*) that were found to greatly exceed the range recorded in the present study. However, values of Zn reported by Oyebisi *et al*. (2012) in *C. amnicola* (0.235 – 2.009*μg/g*) and *Cardisoma armatum* (0.058 – 1.945*μg/g*) were comparatively lower than the range obtained in this study.

CONCLUSION

The carbohydrate, lipid, and fiber contents in soft tissues of *Crassostrea gasar* was found to be associated with the size (shell length, *mm*) of the oyster. Ash, moisture, and protein contents, on the other hand, were not influenced by the size of the animal. For both proximate composition and heavy metal content, higher mean values were generally observed in soft tissues of *C. gasar* in the large-sized oysters compared to the small-sized and medium-sized oysters. The size of C. gasar, therefore, plays a key role in determining

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the food value and uptake of heavy metals by tissues of oysters of the Crassostrea genus. In general, the chemical profile of *C. gasar* was found to be related to size. The large-sized oysters will, therefore, provide better sources of energy and contribute more to food quality (sweetness, appearance, and textural characteristics) compared to the small-sized and medium-sized oysters. On a size-specific basis, the uptake of heavy metals (Pb and Zn) will probably occur more in large-sized, compared to small-sized and medium-sized oysters. However, the levels of heavy metals recorded in soft tissues of *C. gasar* in this study were generally very low and fall within background levels expected in tissues of aquatic biota.

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