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# Proximate Composition and Heavy Metal Content of Three Class Sizes of the Clam *Senilia senilis* from the Andoni River, Niger Delta, Nigeria.

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**Abstract:** The proximate composition and heavy metal content of the clam Senilia senilis from the Andoni River was investigated. Samples were categorized into three class sizes: small (< 30 mm), medium (30-50 mm), and large (> 50 mm) each with 10 sampling units for each class size. Differences across class size were significant for carbohydrate, protein, lipid, and fiber contents but not ash and moisture contents. Effect of class size on proximate composition of soft tissues of clams was more pronounced in carbohydrate and lipid contents relative to protein and fiber contents, with mean values of carbohydrate and lipid generally higher in large-sized clams compared to small-sized and medium-sized clams. Among the heavy metals, variations in mean Cu and Zn levels in S. senilis were minimal compared to Cd and Pb in higher trophic levels of the food chain are higher following consumption of large-sized clams, since the concentrations of these heavy metals appear to be higher in large-sized clams compared to small-sized clams compared to small-sized clams. Find the food samples were metals were and medium-sized clams. Since the concentrations of these heavy metals appear to be higher in large-sized clams compared to small-sized and medium-sized and medium-sized individuals. **Keywords:** Proximate, Composition, Heavy Metal, & Clams

# I. Introduction

Nigeria's endowment with a maritime area of  $46,300 \text{ km}^2$ , an Exclusive Economic Zone (EEZ) area of 210,900 km<sup>2</sup> and inland waters of 12.5 million ha clearly indicates that the country has vast potentials for fisheries development (Woke *et al.*, 2016). Despite this, the current production level of fish of 400,000 metric tons in Nigeria is at a 50% deficit to meet the country's increasing fish needs per annum of at least 1.5 million metric tons. At the root of this problem is Nigeria's growing population which is increasing at an exponential pace and is currently estimated to be approximately 170 million people. Consequently, fishery researchers in Nigeria are beginning to turn their attention to additional sources of protein other than fish, including researches conducted on the nutritional values of the African bull frog *Pyxicephalus adspersus* (Daniel *et al.*, 2016), mangrove oysters *Crassostrea gasar* (Woke *et al.*, 2016), and the crab *Uca tangeri* (Udo and Arazu, 2012).

Among environmental pollutants, metals are of particular concern due to their potentially toxic effect and ability to accumulate in the tissues of aquatic biota (Censi *et al.*, 2006). Aquatic biota take up heavy metals directly from water, sediment and food with concentrations in tissues sometimes reaching levels higher than those detected in the ambient environment (Labonne *et al.*, 2001). Amisah *et al.* (2009) reported mean values of zinc as high as  $23\mu g/g$  and  $42\mu g/g$  in soft tissues of small-sized clams (*Galatea paradoxa*) from the Volta Estuary, Ghana. Murtala *et al.* (2012) investigated bioaccumulation of cadmium, nickel, chromium, cobalt and lead in fish samples of *Hydrocynus forskahlii*, *Hyperopsis bebe occidentalis* and *Clarias gariepinus*) from Ogun Estaury, Nigeria while Dimari and Hati (2009) reported mean cadmium values of 0.09  $\pm$  0.06  $\mu g/g$  (*Tilapia gallier*) and 0.10  $\pm$  0.05  $\mu g/g$  (*Clarias lazera*) in intestines of fish samples from Lake Alau in South Eastern Maiduguri, Nigeria.

Different species of organisms used in biomonitoring have been shown to accumulate heavy metals at different rates (Adeyeye *et al.*, 1996). Bivalves for instance are effective biomonitors and have been widely used for purposes of heavy metal monitoring in various studies conducted around the globe (Phillips and Yim, 1981; Ferreira *et al*, 2004; Otchere, 2003; Woke *et al*, 2016). Like all bivalves, *Senilia senilis* can also accumulate heavy metals in their tissues at concentrations which may be higher than those measured in the ambient environment (El Shenawy, 2002).

Over the last decade, studies have been conducted to investigate the proximate composition and heavy metal content of a wide variety of commercially important and edible aquatic fauna. Some of these studies include investigations on tilapia (Bombata-Fashina *et al.*, 2013), edible frog (Cagiltay *et al.*, 2014; Daniel *et al.*,

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2016), and oysters (Woke *et al.*, 2016). At the Andoni River in the Niger Delta, *S. senilis* are harvested and consumed regardless of their sizes. The sizes captured and sold at the Kaa Waterside Market was found to range from <30mm to >50mm. Woke *et al* (2016) have shown that different class sizes of the bivalve *Crassostrea gasar* accumulate heavy metals in varying quantities. Among the indigenous people of the Niger Delta, there are size-specific preferences in consumption of clams. It is, however, unclear which class size of *S. senilis* accumulates greater quantities of heavy metals in their soft tissues. The present study, therefore, focuses on the proximate composition and heavy metal content (cadmium, copper, lead, and zinc) of the clam *Senilia senilis* from the Andoni River, Niger Delta, Nigeria. This marine bivalve was chosen for this study because it is a delicacy which features prominently in diets of Niger Delta and is widely sold in many fish markets in Nigeria.

## Study Design

## II. Materials And Methods

The Kaa Waterside Market lies in latitude 4° 34.18'N and longitude 7° 22.10'E in Khana Local Government Area of Rivers State, Southern Nigeria and represents an important landing site of a wide variety of fin- and shell-fishes caught by local fishermen from the Andoni River, Rivers State, Nigeria. Although there appears to be some obvious size-specific preferences in the consumption of clams in the Niger Delta, there is a dearth of information on the class size of *S. senilis* which accumulates greater quantities of heavy metals in soft tissues. In this study, different class sizes of the clam *Senilia senilis* from the Andoni River 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).

## **Collection of Samples**

Clams are well known as seafood, sources of animal protein, and are commonly sold in most fish markets in Nigeria, especially those located close to coastal areas. On January 5, 2015, samples of the clam *Senilia senilis* from the Andoni River were purchased from fish sellers in landing sites at the Kaa Waterside Market to reflect the three dominant groups or class sizes of small- (< 30 *mm*), medium- (30 – 50 *mm*), and large-sized (> 50 *mm*) individuals found in the natural population of clams in the Andoni River (Table 1). Over 15 identical sampling units (i.e. similar shell length) were collected in each of the nominal categories of small-, medium-, and large-sized clams.

## Laboratory Analyses

In the laboratory, samples of *S. senilis* were first cleansed to remove mud and any debris and then washed with distilled water. Samples were then categorized into three class sizes each with 10 sampling units for the small (<30 mm), medium (30-50 mm), and large (> 50 mm) class size categories, based on shell length (mm) (Table 1). Soft tissues of *S. senilis* were extracted from their shells using a sterile stainless steel knife as recommended in previous studies (Koh *et al.*, 2011; Madkour *et al.*, 2011; Woke *et al.*, 2016). Soft tissues of *S. senilis* were dried by thinly spreading them in a hot-box oven at a temperature of 105°C for 48 h (Fashina-Bombata and Megbowon, 2012; Woke *et al.*, 2016), 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.

<b>Table 1</b> Mean class size distribution of the clam <i>Senilia senilis</i> (mean $\pm$ SE, n = 10) caught by artisanal
fishermen from the Andoni River.

	Shell Length $(n = 10)$			
	Small (< 30 <i>mm</i> )	Medium (30 - 50 mm)	Large (> 50 mm)	
Mean	30.91	43.85	65.27	
SE	1.12	0.66	1.16	

## **Proximate Composition**

Proximate analysis was carried out in 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  $500^{\circ}$ C for 3 h. Moisture content was determined by heating 1g oven-dried finely ground sample in a hot box oven at  $105^{\circ}$ 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<sub>4</sub>), 5ml nitric acid (HNO<sub>3</sub>) and 0.5ml sulphuric acid (H<sub>2</sub>SO<sub>4</sub>). 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. Descriptive statistics (means and standard errors) were used to depict trends in proximate composition and heavy metal content 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).

## **III.** Results

#### **Proximate Composition**

Results showing means and standard errors of ash content of *Senilia senilis* from the Andoni River are presented in Table 2 below. A slight variation was observed in ash content which ranged from a minimum in small-sized clams  $(2.32 \pm 0.16\%)$  to a maximum in large-sized clams  $(3.48 \pm 0.28\%)$ . A similar pattern was observed in moisture content of the bivalves, with large-sized clams recording mean moisture content value  $(4.65 \pm 0.33\%)$  that was slightly higher than that accounted for by small-sized individuals  $(3.29 \pm 0.45\%)$ . These slight variations in ash and moisture contents of *S. senilis* were, however, not found to be significant by results of ANOVA (p > 0.05, Table 3).

On the other hand, obvious differences in carbohydrate, protein, lipid, and fiber contents were observed in soft tissues of *S. senilis* in which mean values generally increased from a minimum in small-sized clams to peak values in large-sized individuals (Table 2). The carbohydrate content of large-sized clams was nearly 10 times as high as that of small-sized clams while that of medium-sized clams was at least 7 times higher than that of small-sized individuals. The result of ANOVA on carbohydrate content of *S. senilis* was found to be significant ( $F_{2,3} = 476.386$ , p < 0.05, Table 3). There was significantly higher carbohydrate content in largesized clams compared to small-sized clams (Tukey, p < 0.05).

A similar range of mean values were also observed in protein  $(28.06 \pm 0.34 - 39.51 \pm 0.19\%)$ , lipid  $(2.14 \pm 0.03 - 5.55 \pm 0.07\%)$ , and fiber  $(9.47 \pm 0.29 - 14.36 \pm 0.24\%)$  in which mean content generally increased from minimum values in small-sized individuals to peak values in large-sized individuals (Table 2). ANOVA found differences which were significant in mean content of protein ( $F_{2,3} = 343.82$ , p < 0.05), lipid ( $F_{2,3} = 1087.757$ , p < 0.05), and fiber ( $F_{2,3} = 55.874$ , p < 0.05) (Table 3). For these parameters, there were significantly higher content in large-sized clams, compared to small-sized clams. Medium-sized clams showed mean protein, lipid, and fiber content values that were significantly higher than small-sized clams but not large-sized clams (Tukey, p < 0.05).

**Table 2** Proximate composition (mean  $\pm$  SE, n = 2) of tissues of 3 class sizes of Senilia senilis from the Andoni<br/>River.

Class Size		Proximate Compo	Proximate Composition (%)			
	Ash	Moisture	Carbohydrate	Protein	Lipid	Fiber
Small	2.32±0.16	3.29±0.45	4.33±0.12	28.06±0.34	2.14±0.03	9.47±0.29
Medium	3.00±0.24	3.32±0.15	11.36±0.08	31.92±0.38	4.22±0.04	12.02±0.42
Large	3.48±0.28	4.65±0.33	14.22±0.38	39.51±0.19	5.55±0.07	14.36±0.24

Table 3 One-way analysis of variance on proximate com	position (%) of Senilia senilis from the Andoni River.
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Composition	df	MS	F	P-value
Ash	2	0.679	6.208	> 0.05
Moisture	2	1.211	5.415	> 0.05
Carbohydrate	2	51.847	476.386	< 0.05*
Protein	2	67.870	343.820	< 0.05*
Lipid	2	5.892	1087.757	< 0.05*
Fiber	2	11.939	55.874	< 0.05*
*Significant at p < 0.05				

## Heavy Metal Content

The means and standard errors of cadmium (Cd) content in soft tissues of *Senilia senilis* are presented in Table 4. Mean Cd values ranged from a minimum of  $0.04 \pm 0.00 \ \mu g/g$  (small-sized clams) to a maximum of  $0.11 \pm 0.01 \ \mu g/g$  (large-sized clams). ANOVA on Cd was significant (F<sub>2,3</sub> = 129.00, p < 0.05, Table 5). There was significantly higher Cd content in large-sized clams compared to small-sized clams (Tukey, p < 0.05).

Mean copper (Cu), lead (Pb), and zinc (Zn) contents in soft tissues of S. senilis showed similar pattern as Cd across the three class sizes in which mean values also varied from a minimum in small-sized clams to a maximum in large-sized individuals. The range of mean values obtained for Cu, Pb, and Zn were  $8.03 \pm 0.19 - 16.39 \pm 0.11 \ \mu g/g$ ,  $0.09 \pm 0.00 - 0.93 \pm 0.02 \ \mu g/g$ , and  $20.55 \pm 0.18 - 25.22 \pm 0.20 \ \mu g/g$  respectively (Table 4). Results of ANOVA on Cu (F<sub>2,3</sub> = 264.033), Pb (F<sub>2,3</sub> = 1352.167), and Zn (F<sub>2,3</sub> = 216.740) were all significant (p < 0.05, Table 5). In a decreasing order of magnitude, the concentrations of the four heavy metals followed the trend: *large-sized clams > medium-sized clams > small-sized clams*. The concentrations of the various heavy metals in medium-sized clams were found to be significantly higher than values recorded in small-sized clams, but lower compared to those large-sized clams (Tukey, p < 0.05).

**Table 4** Concentrations (mean  $\pm$  SE, n = 2) of heavy metals ( $\mu g/g$ ) in tissues of 3 class sizes of *Senilia senilis* from the Andoni River.

Class Size		Heavy Metal Cont			
	Cd	Cu	Pb	Zn	
Small	$0.04 \pm 0.00$	$8.03 \pm 0.19$	$0.09 \pm 0.00$	$20.55 \pm 0.18$	
Medium	$0.08\pm0.00$	$11.71 \pm 0.39$	$0.22 \pm 0.01$	$23.37 \pm 0.05$	
Large	$0.11\pm0.01$	$16.39\pm0.11$	$0.93\pm0.02$	$25.22\pm0.20$	

Table 5 One-way analysis of variance on heav	y metal content ( $\mu g/g$ ) of <i>Senilia senilis</i> from the Andoni River.
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Heavy Metal Content	df	MS	F	P-value
Cd	2	0.002	129.000	< 0.05*
Cu	2	35.068	264.033	< 0.05*
Pb	2	0.406	1352.167	< 0.05*
Zn	2	11.086	216.740	< 0.05*
*Significant at $p < 0.05$				

## IV. Discussion

The results of ash content of Senilia senilis obtained in this study were in agreement with Woke et al. (2016) who reported mean values of ash content ranging from 2.63 - 3.07% in the mangrove oyster Crassostrea gasar from the Andoni River. Daniel et al. (2016) also reported a range of mean values in Pyxicephalus adspersus  $(4.02 \pm 0.51 - 5.98 \pm 0.61\%)$  and Oreochromis niloticus  $(4.02 \pm 0.51 - 5.98 \pm 0.61\%)$  from ARAC in Aluu, Port Harcourt, that were consistent with the range of values recorded in clams in the present study. The mean moisture content of S. senilis recorded were several orders of magnitude lower than the values reported by Daniel et al. (2016) in Pyxicephalus adspersus (71.86  $\pm$  0.51 – 78.47  $\pm$  1.65%) and Oreochromis niloticus  $(77.03 \pm 0.66 - 78.95 \pm 3.44\%)$  but similar to the range of 2.57 - 3.59% found in oysters (Woke *et al.*, 2016). The mean values of carbohydrate recorded in clams in this study were considerably lower than the range of 49.68 – 58.39% reported by Woke et al. (2016) in C. gasar from the Andoni River but higher than mean values observed by Daniel et al. (2016) in P. adseprsus ( $0.54 \pm 0.01 - 0.64 \pm 0.26\%$ ) and O. niloticus ( $1.12 \pm 0.09 - 0.09 \pm 0.000$ )  $1.29 \pm 0.10\%$ ). However, results of this study were congruent with Swapna and Ravinder (2015) who also reported a similar range of mean carbohydrate values (4.21 - 15.67%) in the bivalve Parreysia cylindrical. Higher mean values of protein were recorded in S. senilis in the current study compared to P. adspersus and O. *niloticus* in which mean values varied from  $6.41 \pm 0.46 - 12.04 \pm 0.87\%$  and  $9.03 \pm 2.32 - 13.53 \pm 0.74\%$ respectively (Daniel et al., 2016).

Mean values of Cd recorded in soft tissues of *S. senilis* were in agreement with the range of values reported by Dimari and Hati (2009) in the intestines of *Tilapia gallier* ( $0.09 \pm 0.06 \mu g/g$ ) and *Clarias lazera* ( $0.10 \pm 0.05 \mu g/g$ ) from Lake Alau, Maiduguri, Nigeria while mean *Cu* values obtained were lower than the range of  $25.06 \pm 1.56 - 31.28 \pm 0.78 \mu g/g$  reported in tissues of *C. gasar* (Woke *et al.*, 2016). Mean Pb values recorded in soft tissues of clams in this study were congruent with Woke *et al.* (2016) who also reported a similar range of values in oysters ( $0.20 \pm 0.02 - 0.53 \pm 0.02 \mu g/g$ ) from the same study area. Dimari and Hati (2009) also reported mean Pb values in gills ( $0.48 \pm 0.11 - 0.53 \pm 0.04 \mu g/g$ ), stomach ( $0.35 \pm 0.20 - 0.40 \pm 0.02 \mu g/g$ ), intestines ( $0.12 \pm 0.01 - 0.31 \pm 0.16 \mu g/g$ ), and liver ( $0.32 \pm 0.12 - 0.40 \pm 0.03 \mu g/g$ ) of samples of *Tilapia gallier*, *Clarias lazera*, and *Heterotis niloticus* from Lake Alau, Maiduguri, Nigeria. In a study conducted in Volta Estuary, Ghana, Amisah *et al.* (2009) reported mean Zn concentrations in the range  $23 - 42 \mu g/g$  in small-sized clams *Galatea paradoxa* from the Ada sampling station which were found to be similar to the range of mean values recorded in clams in the present study. However, mean values of Zn recorded in this

study were slightly lower than the range  $(28.89 \pm 0.53 - 42.69 \pm 1.81 \ \mu g/g)$  reported in the mangrove oyster *C*. *gasar* (Woke *et al.*, 2016).

Taste, appearance, texture, and stability are the quality indicators of sea foods and many other foods. Ash content as well as mineral content provide measures of quality of sea foods. Whereas ash content measures the total amount of minerals present within a sample, mineral content provides a measure of the amount of specific inorganic components present within a sample. Carbohydrates also contribute greatly to the quality (sweetness, appearance, and textural characteristics) of many foods. Carbohydrate and lipid are known to contribute to good food quality. The carbohydrate and lipid contents of clams recorded in this study were thrice greater than those of small-sized clams. It is, therefore, not improbable that large-sized clams will provide better food quality compared to small-sized and medium-sized clams. Size does not seem to affect protein and dietary content of *S. senilis* particularly when the comparison is made between medium-sized and large-sized class.

Variations in Cu and Zn contents of S. senilis across class size was minimal compared to Cd and Pb. Despite the relatively low content of Pb recorded in this study, amount of Pb in soft tissues of large-sized clams was 10 times higher than that recorded in small-sized clams. On the other hand, Cu content of large-sized clams were only 3 times higher than values obtained in small-sized individuals. Large-sized clams, therefore, have the potential to accumulate higher concentrations of heavy metals in their tissues compared to small-sized and medium-sized individuals. Amisah *et al.* (2009) also observed relatively higher Zn concentrations in large-sized individuals of the clam *Galatea paradoxa* compared to small-sized individuals. In a study conducted to investigate the effects of clam size on heavy metal accumulation in soft tissues of *G. paradoxa* from the Volta Estuary, Ghana, these workers also reported mean Zn values in large-sized clams that were nearly twice values obtained in small-sized clams during the month of July at the Ada sampling station. Even at very low concentrations, there is considerable variation in heavy metal content of small-sized and large-sized clams. However, the relative significance of these variations in heavy metal content across class size are generally metal-specific as we have seen in results of this study.

#### V. Conclusion

Clams (*Senilia senilis*) caught by artisanal fishermen at the Andoni River generally fall into three class size distributions: small size (<30 mm), medium size (30 - 50 mm), and large size (>50 mm). Effect of class size on proximate composition of soft tissues of clams from the Andoni River was more pronounced in carbohydrate and lipid contents but to a small extent in protein and fiber contents. Evidence was lacking that the observed variations in ash and moisture contents of *S. senilis* could be attributed to differences in class sizes of the animals. Mean values of carbohydrate and lipid were generally higher in large-sized clams compared to small-sized and medium-sized clams. The amounts of carbohydrate and lipid in tissues of clams at the Andoni River were, therefore, size-specific.

This was also true of concentrations of Cd and Pb in soft tissues of the animals. Among the heavy metals, variations in mean Cu and Zn levels in soft tissues of S. senilis were minimal compared to Cd and Pb in which large-sized clams also had mean values that were several orders of magnitude higher than small-sized clams. Carbohydrate and lipid are known to contribute to good food quality. The relatively higher amounts of carbohydrate and lipid contents in large-sized clams will invariably suggest better food quality compared to small-sized and medium-sized clams. However, there may also be the cumulative risk of Cd and Pb uptake in higher trophic levels of the food chain following consumption of large-sized clams, since the concentrations of these heavy metals appear to be higher in soft tissues of large-sized clams compared to small-sized and medium-sized individuals.

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