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Full Length Research

Effects of clam size on heavy metal accumulation in whole soft tissues of *Galatea paradoxa* (Born, 1778) from the Volta estuary, Ghana

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The Volta basin clam, *Galatea paradoxa,* is collected for food and remains an important affordable protein source for the riparian communities in the catchment. Clams accumulate metals in their soft tissues, which can be toxic to humans when consumed. A study was, therefore, carried out to examine the concentrations of Mn, Zn, Fe and Hg in *G. paradoxa*, at 3 different size classes: small (20 - 40 mm), medium (41 – 60 mm) and large (>60 mm) at Ada and Aveglo in the Volta estuary area in Ghana. The concentrations of heavy metals in the clams varied considerably between the two locations. There were, however, no significant differences ($p > 0.05$) in Mn, Fe and Zn concentrations among the different size classes, indicating a similar bioavailability of the metals at both locations and, possibly, an efficient metabolism to keep the concentrations of Mn, Fe and Zn relatively similar. Mercury concentrations in the Ada clams varied significantly ($p < 0.05$) among the different size classes. A Risk Analysis indicated that the concentrations of heavy metals in the clams were within acceptable limits and safe for human consumption.

Key words: *Galatea paradoxa*, Volta estuary, heavy metals, size classes.

INTRODUCTION

Heavy metals occur in aquatic environments from natural processes and anthropogenic activities (Connell et al., 1999; Franca et. al., 2005). The contamination of natural waters by heavy metals affects aquatic biota and poses considerable environmental risks and concerns (Cajaraville et al., 2000; Ravera, 2001; Otchere, 2003) and human health.

Contaminants can persist for many years in sediments, where they hold the potential to affect human health and the environment (Mackevičiene et al., 2002). The analyses of water or sediment samples, however, are subject to a variety of shortcomings, in that the methods do not allow for the estimation of the quantity of the metal which is biologically available (Etim et al., 1991). It is against this background that bio-indicators are preferred in environmental monitoring. Bivalves are effective biomonitors and have been widely used for heavy metal monitoring purposes worldwide (Phillips and Yim, 1981, Etim et. al., 1991, Ferreira et al., 2004, Otchere, 2003, Tay et al., 2004). Heavy metal accumulation in bivalves is influenced by several factors (Phillips and Rainbow, 1994). Some of these include seasonality (Regoli, 1998), location (Blackmore and Wang, 2003) salinity (Chong and Wang, 2001), organic matter (Pan and Wang, 2004), sex (Sokolowski et al., 2003), food acquisition capability (Saavedra et al., 2004), stage of gonadal development (Bryan et al., 1980) and size-weight relationships (Phillips, 1976, Riget et al., 1996).

The Volta estuary clam, *Galatea paradoxa* (Born, 1778) is a commercially important bivalve in Ghana exploited mainly for its flesh. Like all bivalves, the organism can accumulate heavy metals in their tissues at concentrations in excess of the ambient water (El Shenawy, 2002).

The Volta basin has inherited a legacy of pollution from metal fabrication and agricultural industries for several decades. In particular, the estuary at Ada and Aveglo has been implicated as the most impacted sites receiving discharges from heavy metals sources.

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At the Volta estuary Ghana, *G. paradoxa* are harvested and consumed regardless of their sizes. Frequently, the sizes captured range from a minimum of about 20 mm to over 60 mm. Anecdotal information suggests that the various sizes of clams may accumulate heavy metals in varying quantities. While some consumers prefer small clams others prefer large or medium sized clams for their dishes. It is unclear which sizes of the clams have the tendency to bio-accumulate heavy metals in greater quantities in soft tissues. This study was, therefore, conducted to assess the concentrations of some heavy metals in the soft tissues of clams in relation to selected categories of clam body sizes.

MATERIALS AND METHODS

Study area

The Volta Lake was created out of the Volta River to, primarily, provide hydroelectricity for Ghana. The Volta is the largest man-made lake in the world and the river takes its source from a low range of hills in Bobo Dioulasso in Burkina Faso. It then flows through northwestern Ghana to the east where it enters the sea at Ada. Intense clam fishing activities take place at the lower estuary at Ada and in the upper reaches of the estuary at Aveglo.

The study was carried out at Ada and Aveglo, at the Volta Estuary, in Ghana, from March 2008 to September 2008. Ada located 05°49' 18.6" N and 000°38.46' 1"E and Aveglo 05°53 28.2" N and 000° 38' 24.7"E represent the most active clam fishing grounds of the Volta Estuary.

Collection and processing of clam samples

Clam samples were obtained from fishermen's catch at bi-weekly intervals for 7 months from March to September, 2008. The samples were transported, partly submerged in river water, to the laboratory, in thermally insulated chests within 12 h for processing prior to analyses. In the laboratory, clam samples were cleansed to remove mud and debris and subsequently washed with double distilled water. For each sampling location 40 individual clams for each size class were obtained and categorized based on shell length as: small (20 - 40 mm), medium (41 - 60 mm) and large (>60 mm). There were 3 replicates for each size class.

A sterile stainless steel knife was used to quickly dislodge and remove the soft tissue or flesh of each clam from the shell as described by Chiu et al. (2000). The flesh of each subsample was oven-dried to a constant weight at 60°C for 72 h (Ferreira et al., 2004, Parlak et al., 2006). Each dry clam sample was weighed to the nearest 0.0001 g. Clams of each size class were ground together into fine powder using a porcelain pestle and mortar. Homogenised subsamples were stored in air-tight, acid-washed (0.1 M HCl) snap-top glass vials with plastic caps for heavy metals analyses (Environmental Agency, 2008).

Digestion of clam samples and heavy metal determinations

Digestion of samples was done in accordance with procedures adopted in Jin et al., 1999; Sastre et al., 2002; Otchere, 2004 and filtered with a Whatmann Glass Microfibre filter paper before allowing to cool at room temperature. The filtrate was diluted to 50 ml in volumetric flasks with double distilled water prior to analysis. Total concentrations of zinc, iron and manganese were determined using a Buck Scientific Model VGP flame Atomic Absorption Spectrophotometer (AAS). The Atomic Mercury Analyzer (Model HG 5000) equipped with a mercury lamp at a wavelength 253.7nm was used for the determination of total mercury in the clam soft tissue samples. For each metal analysis there were 3 replicate samples

Hydrographic factors

Bi-weekly measurements of temperature, pressure, salinity, pH, Total Dissolved Solids (TDS), conductivity and Dissolved Oxygen (DO) were taken *in-situ* at both sampling sites over the study period using a Hanna (HI 9028) multi-parameter probe.

Assessment of the health risk associated with the consumption of clams from ada and aveglo

The environmental health risk associated with the consumption of G. *paradoxa* was assessed by making a comparison between environmental status, represented by the concentrations of heavy metals in the clams and threshold values which may cause adverse effects in human consumers.

Risk quotient (RQ) was calculated as the ratio between concentration of heavy metal in the clam and the Level of Concern (LOC) for that metal (Fung et al., 2004). A Level of Concern (LOC), which is a threshold concentration of a chemical above which a hazard to human health may exist, was calculated as the ratio of Tolerable Daily Intake (TDI) and the Rate of Shellfish Consumption (RSC) (Fung et al., 2004). For the purpose of this study, it was assumed that total trace metal exposure was derived solely from *G. paradoxa* consumption.

Data on average national rate of shellfish consumption (RSC) of Ghana was calculated from the Daily Food Supply per capita from Fish and Fishery Products of the FAO (FAOSTAT, 2004- http://apps.fao.org

Statistical analysis

Data on the heavy metal analyses were subjected to a one-way Analysis of variance (ANOVA) to test for significant differences ($p < 0.05$) in the concentrations of the heavy metals for the different sizes classes of the clams at the two locations. Graphs were generated using the GraphPad Prism 5 Software.

RESULTS

Hydrographic parameters

At Ada, pH declined marginally from 6.99 in April to 6.48 in September. Temperature remained fairly stable at 29.22°C in May and 27.28°C in September. Dissolved Oxygen (DO) concentrations were relatively high in March (8.76 mg l^{-1}) but dropped to (2.48 mg l^{-1}) in September. Salinity was, however, remained constant at 0.03 throughout the sampling period. Total Dissolved Solids (TDS) remained fairly constant with values ranging between 31 and 35 over the study period. Conductivity values were low, ranging from 60 μScm^{-1} to a maximum of 70 $\mu S\ cm^{-1}$ (Table 2a) for Ada and between 63 μScm^{-1} and 84 μScm^{-1} for Aveglo (Table 1). At Aveglo, pH was 7.08 in June and 6.89 in September. Temperature remained fairly constant at during the sampling period (Table 1). DO concentrations at

Table 1 (a). Hydrographic parameters of the Volta Estuary at Ada (a) and Aveglo (b), March – Sept, 2008

Parameter	March	April	May	June	July	Aug	Sept
pH	6.99	7.05	6.63	6.99	6.55	6.99	6.48
Temperature (℃)	28.16	28.01	29.22	28.49	28.08	27.38	27.28
Pressure (mmHg)	766	765	770	769.5	769.6	770	768.7
Salinity	0.03	0.03	0.03	0.03	0.03	0.03	0.03
TDS (mg/l)	31	30	31	33	35	33	32
Dissolved Oxygen(mg/l)	8.76	7.21	5.84	6.33	3.16	3.06	2.48
Conductivity (µs/cm)	62	60	61	66	70	65	63

Table 1(b)

pH	7.00	7.04	7.06	7.08	7.00	6.90	6.89
Temperature (℃)	28.20	28.30	28.35	28.49	28.11	27.33	27.19
Pressure (mmHg)	767.3	767.4	767.3	768.4	769.2	769.1	768
Salinity	0.03	0.03	0.03	0.03	0.04	0.03	0.03
TDS (mg/l)	32	32	33	34	42	33	32
Dissolved Oxygen (mg/l)	6.77	6.78	6.79	6.78	3.16	2.70	2.38
Conductivity (µs/cm)	70	70	69	68	84	66	63

Table 2. Risk analysis for minimum and maximum concentrations of metals present in *Galatea paradoxa* from Ada and Aveglo in the Volta estuary, Ghana.

Metal	Min. Conc. (µg/g)	Max. Conc (µg/g)	TDI or ESADDI (µg/g/d)	RSC LOC$_1$ (g/p/d) (µg/g)	LOC$_2$ (µg/g)	1-For LOC$_1$	2-For LOC$_2$
THg	0.028	0.074	33 - 43[a]	0.95 34.74	45.26	0.0021	0.0016
Zn	13	49	5600 - 15000[b]	0.95 5894.74	15789.47	0.0083	0.0031
Fe	79	539	8000 - 45000[c]	0.95 8421.05	47368.42	0.0064	0.011
Mn	49	867	2000 - 11000[c]	0.95 2105.26	11578.95	0.41	0.075

Legend:
TDI-Tolerable Daily Intake (in µg/person/day)
ESAADI-Estimated Safe and Adequate range of Daily Dietary Intake concentrations (in µg/person/day) for all foods set by the National Research Council of the National Academy of Sciences of the USA
RSC- Rate of Shellfish Consumption for Ghana calculated from the Daily Food Supply per capita from Fish and Fishery Products of the FAO (FAOSTAT, 2004- http:// apps.fao.org)
LOC$_1$-Concentration of Consumption (in µg/g) calculated from the lowest value of TDI or ESADDI range
LOC$_2$-Concentration of Consumption (in µg/g) calculated from the highest value of TDI or ESADDI range
RQwcs- Risk Quotient for worst-case scenario: 1-For lowest value of TDI or ESADDI range
2-For highest value of TDI or ESADDI range
[a]- Provisional Tolerable Daily Intake of total mercury; set by FAO/WHO.
[b]- Dietary reference value for zinc; WHO, 2001
[c]- Tolerable Daily Intake of Iron and Manganese; set by the Institute of Medicine of the USA, 2003

Aveglo ranged from of 2.38 mg l^{-1} to 6.78 mg l^{-1} whilst TDS values were between 32 and 42 during the period. Salinity remained constant at 0.03 but with a marginal increase to 0.04 in July (Table 1). Pressure at both locations remained fairly constant.

Heavy metals in whole clam tissues in relation to sizes

Manganese, Zinc, Iron and Total Mercury (THg) concentrations in the clams at Ada and Aveglo are presented (Figure 2). The concentrations of the heavy metals appeared to follow a sporadic pattern with respect to the various sizes and locations (Figure 1a - h)

Manganese concentrations

Manganese (Mn) concentration in the whole soft tissue of the small-sized clams at Ada varied from 73 µg/g in June to 867 µg/g in July. The medium-sized clams (shell lengths of 41 - 60 mm) recorded manganese values of between 68

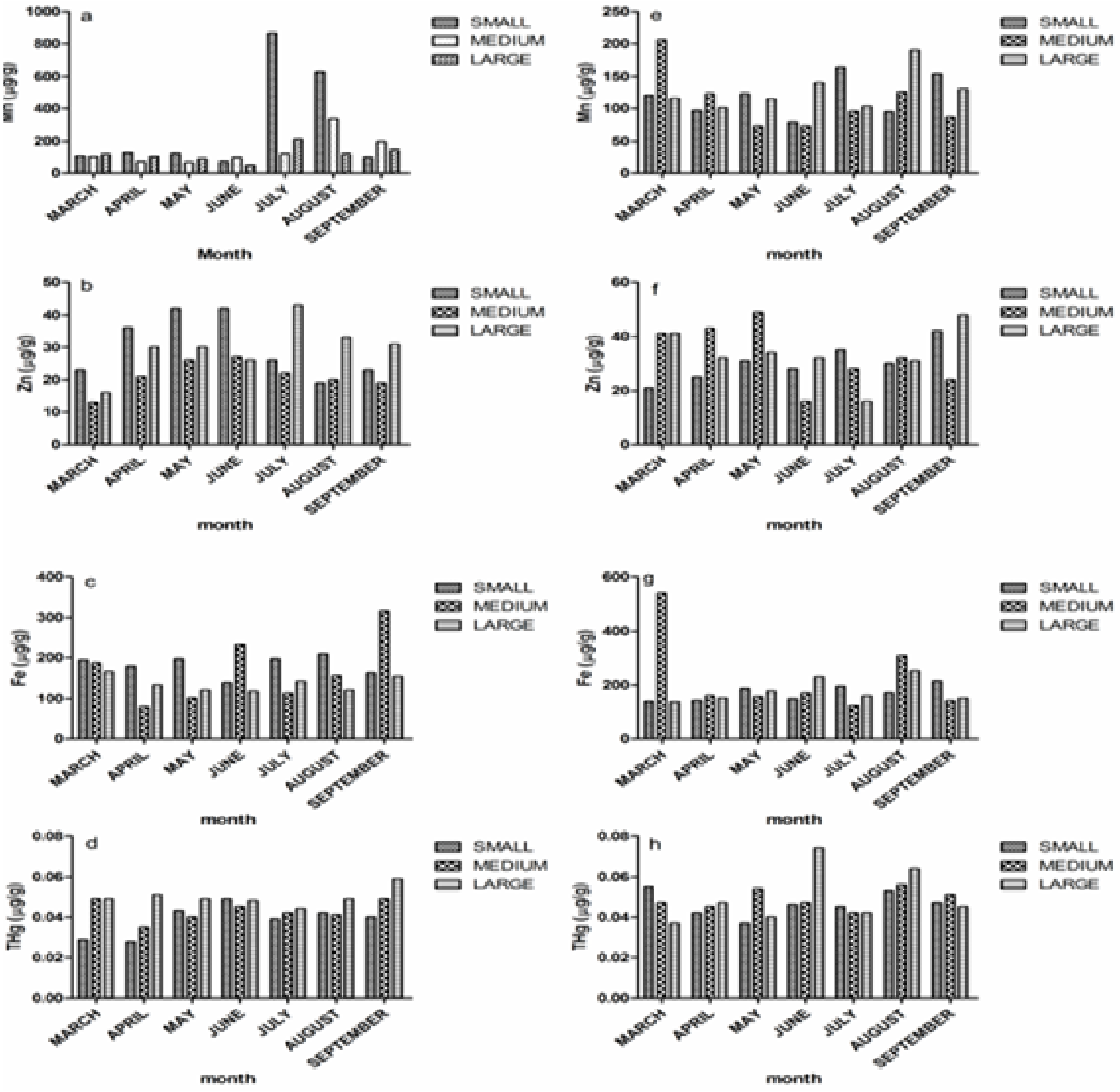

Figure 1. Heavy metal concentrations in soft tissues of the different size classes of *Galatea paradoxa* from Ada (a - d) and Aveglo (e - h) in the Volta estuary in Ghana.

µg/g in May 2008 and 336 µg/g in August 2008. Manganese concentrations in the tissues of the large-sized clams (above 60 mm) ranged from 49 µg/g in June to 212 µg/g in July (Figure 1a).

At Aveglo, Mn concentrations in the tissues of the small-sized clams showed a sharp decline from 120 µg/g in March to79 µg/g in May. It, however, peaked in June up to 164 µg/g coinciding with the onset of the spawning season of the clam (Figure 1e).

Manganese concentrations increased from 73 µg/g in May to 125 µg/g in August (Figure 1e). A peak value of 206 µg/g was, however, recorded in March, the onset of the major rainy season in Ghana. Large-sized clams exhibited a trend similar to what was observed for the small and medium-sized clams, suggesting similar regulating mechanisms of manganese in the tissues of clams regardless of size.

Zinc concentrations

At Ada, zinc concentrations in small-sized clams were 23 µg/g in March and 42 µg/g in May and June (Figure 1b). The medium-sized clams also exhibited a rise in zinc concentrations from March to June 2008 with values of 13 µg/g and 27 µg/g respectively. There was, however, a declining trend from June (27 µg/g) to September (19 µg/g) (Figure 1b); indicative of a relationship between the accumulation of essential heavy metals and the reproductive cycle of *G. paradoxa,* with concentrations increasing to a peak value coinciding with the onset of the spawning season of *G. paradoxa*. Zinc concentrations in the tissue of the large-sized clams showed a rise from 16 µg/g in March to 43 µg/g in July, a trend similar to those exhibited by the small and medium-sized clams (Figure 1b).

At Aveglo Zn concentrations followed an irregular pattern for small-sized clams. September recorded the highest zinc concentration of 42 µg/g whilst June recorded the lowest value of 28 µg/g (Figure 1f). Temporal variations in the concentrations of zinc in the medium-sized clams followed a non-uniform trend with May recording a peak value of 49 µg/g (Figure 1f). In the large-sized clams, zinc concentrations showed a decline from March to July; thus from 41 - 16µg/g (Figure 1f).

Mercury concentrations

At Ada, Total Mercury concentrations (THg) for the small-sized clams ranged from 0.028 µg/g in April to 0.042 µg/g in August. The medium-sized clams recorded a highest THg value of 0.049 µg/g in March and September whilst a lower value of 0.035 µg/g was recorded in April 2008. THg concentrations ranged between a low of 0.044 µg/g in July and high of 0.059 µg/g in September in the large-sized clams (Figure 1d).

At Aveglo Hg concentration in the tissues of the small-sized clams was 0.055µg/g in March 2008 and 0.037µg/g in May. Medium-sized clams recorded values ranging from 0.042 µg/g in July to 0.056 µg/g in August. The large-sized clams had a low THg concentration of 0.037µg/g in March 2008 and a high of 0.074 µg/g in June 2008 (Figure 1h).

Variations in the mean heavy metal concentrations in the different clam size classes for both sampling stations over the sampling period were not statistically significant ($p<0.05$) except for Hg concentrations in the Ada clams (Figure 2)

Assessment of the health risk associated with the consumption of clams from ada and aveglo

Reference data for the evaluation of risks to human health associated with consumption of the clams containing trace metals are presented (Table 2). The concentrations indicated for total Mercury (THg), Zn, Fe and Mn are well above the concentrations found in the clams of the Volta estuary (Table 2).

DISCUSSION

Trace metal concentrations in clams depend on numerous environmental and biological factors (Cossa, 1989; Kramer, 1994; Kljaković-Gašpić, 2007). Seasonal, intense clam fishing is carried out at Ada and Aveglo during the onset of rains in March and start of the dry season in December. During this season there is considerable run-off from metal fabrication industries in the catchment. It might well be that the elevated concentrations of heavy metals in the clams might originate, partly, from contributions from surface run-off from such locations into the Volta estuary. Earlier studies by Chouba et al. (2007) in Tunisia demonstrated higher concentrations of heavy metals in clams during high rainfall periods and period for most intense fishing activities. The findings from this study appear to agree with those of that study.

The pattern of variation of heavy metal accumulation in whole soft tissues of the clams appears to be influenced largely by the reproductive stage of the organism. Studies by Etim, 1990; and Etim *et. al.,* 1991 revealed that clam spawning starts in June (when mean dry tissue weight maximum occurs), and is completed between October and November (when mean dry tissue weight minimum occurs). Studies have shown that during the spawning period, proteins and carbohydrate contents, which have a high affinity for heavy metals, are accumulated for gonad tissue production, energetic storage and consumption (Latouche and Mix, 1982; Páez-Osuna et al., 1995; Galstoff, 1964; Etim et. al., 1991). For instance, it is observed that the ripe oyster gonad may comprise 31 to 41% of the total body weight. On the basis of this, Cunningham and Tripp (1975) argued that if metals were accumulated in the gonad tissues, an appreciable loss of metal from the body might occur during spawning. The balance between heavy metal contents of gametes that are ejected and the effect of gamete production resulting in tissue dilution of total metal concentrations of whole soft tissue is significant. This, possibly, explains why most of the peak metal concentrations coincided with the onset of the spawning period of the *G. paradoxa.*

Variations in the mean heavy metal concentrations in the different clam size classes for both sampling stations were not statistically significant ($p < 0.05$) except for Hg concentrations in the Ada clams (Figure 2). This could be due to similarities in bioavailability of the heavy metals to the clams (Ferreira et al., 2004) and homogeneity in environmental and hydrographic parameters at the two locations (Table 1).

The high peak Mn concentrations of 867 and 629 µg/g at Ada (Figure 1) for the small-sized clams in the months of July and August as opposed to the relatively lower concentrations in the medium and large clams suggests that the medium and large size classes are sexually-mature and have an efficient metabolism and detoxifying processes (Connell et al., 1999) to keep the concentrations of Mn, Fe and Zn (essential heavy metals) relatively lower. This possibly explains why there was a significant variation ($p < 0.05$) in the concentrations of mercury, a non-essential heavy metal, in the tissues of the Ada clams.

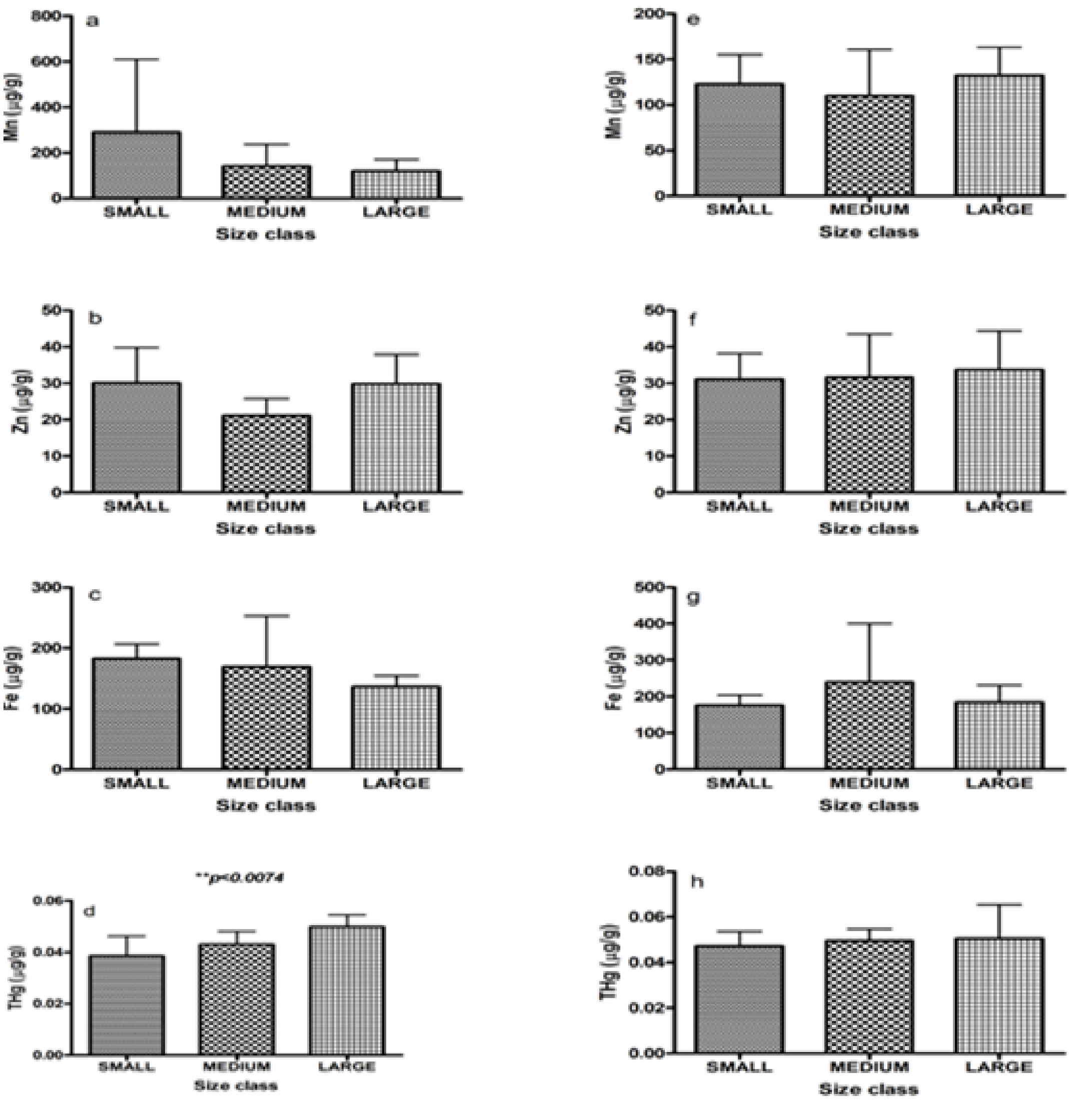

Figure 2. Means ± SD of heavy metal concentrations in the clam size classes from Ada (a - d) and Aveglo (e - h). Significant variations: **p < 0.01.

Essential heavy metals have intracellular regulatory mechanisms to keep their concentrations in equilibrium in the organisms (Luoma and Rainbow, 2008).

The study did not observe any discrete point source of pollution in the confines of the estuary. Thus, even clams in with no known point sources of contamination may have measurable body burdens of heavy metals. This could be due to natural processes as weathering, hydrological conditions, intensive leaching of mineralized rocks or run-off, particulate matter re-suspension and primary production. These processes are highly variable on periodic basis and could, possibly, account for the differences in monthly metal concentrations in the Volta clams. Variability of heavy metal concentrations can also be caused by changes in the physiological conditions of the clams (Phelps et al., 1985; Ferreira et al., 2004) and environmental parameters including temperature, pH, salinity, oxygen concentrations (Phillips, 1976; Luoma and Bryan, 1982). Except Hg, all the metals examined in this study are essential for clams and have intracellular regulatory mechanisms (Luoma and Rainbow, 2008) and equilibrium maintenance in the organisms.

Implications for human consumption of whole soft tissue of clams from the Volta estuary

Analysis of Risks concentrations associated with the consumption of clams revealed that the concentration of the heavy metals found in the clams were within acceptable limits using various indicators such as Tolerable daily Intake (TDI) ESAADI, RSC, Risk Quotients and LOCs Risk

(Table 2). Against this background, the clams can be said to contain acceptable limits of Mn, Zn, Fe and Hg for human consumption.

Conclusions

The Volta clam, *Galatea paradoxa,* accumulates Mn, Zn, Fe and Hg in their tissues, regardless of sizes. Bioaccumulation of Mn and Zn appear to be linked to gonadal recrudescence and are accumulated prior to spawning and in the rainy seasons. There appears to be some regulatory mechanism for relatively large clams to regulate to some extent the concentrations of Mn, Zn and Fe but they do not appear to be able to regulate concentrations of mercury because it is not one of the essential element for gonadal recrudescence. Some environmental factors possibly contribute to the concentrations of the metals. There were, however, significant temporal variations in the concentrations of heavy metals in soft tissues of clams. Despite considerable industrial and other anthropogenic inputs into the Volta estuary, the concentrations of Mn, Fe, Zn and Hg in the Volta clams remain within acceptable limits. *G. paradoxa* in the estuary, therefore, meet the acceptable standards (Table 2) and the clams are safe for human consumption.

It, however, remains important that allochtonous inputs from the catchment area are devoid of any metals and regulatory mechanism being enforced to ensure that current trends are not exacerbated.

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