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Relationship Between Gonad Maturation and Heavy Metal Accumulation in the Clam, *Galatea paradoxa* (Born 1778) from the Volta Estuary, Ghana

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Abstract The relationship between gonadal development and the concentrations of four heavy metals Mn, Zn, Fe and Hg in the tissues of the clam *Galatea paradoxa* was evaluated at the Volta estuary, Ghana, over an 18-month period. Metal concentrations in the clam tissues were highly variable over the sampling period and seemed to be influenced by the reproductive cycle of the clam. Mn concentrations varied over a wide range from 49 to 867 µg/g and exhibited a significant positive correlation with gonadal development ($p = 0.0146$, $r^2 = 0.3190$). Zn and Fe concentrations ranged from 13 to 59 µg/g and 79 to 484 µg/g, respectively and both revealed negative relationships between gonad development and metal accumulation (Zn ($p = 0.0554$, $r^2 = 0.0554$) and Fe ($p = 0.1040$, $r^2 = 0.1567$)). Hg concentrations ranged from 0.026 to 0.059 µg/g over the sampling period and exhibited a slight positive relationship between gonadal development and metal accumulation ($p = 0.0861$, $r^2 = 0.1730$).

Keywords Reproductive cycle · *Galatea paradoxa* · Heavy metals · Volta estuary

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Heavy metal accumulation in bivalves is influenced by several abiotic and biotic factors (Phillips and Rainbow 1994). Some of these factors include: season (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), and the stage of gonadal development and size/weight (Phillips 1976; Riget et al. 1996). These natural factors may influence observed variations in bioaccumulated metals (Phillips and Rainbow 1994). Seasonal variations in heavy metal concentrations in animal tissues have also been related to a great extent to seasonal changes in flesh weight during development of gonadic tissues (Cossa and Rondeau 1985; Joiris et al. 1998; Otchere et al. 2000, 2003) because it is believed that prior to the spawning period, there is the production and storage of compounds (proteins and glycogen) which have a high affinity for heavy metals in the gonad tissue (Latouche and Mix 1982; Páez-Osuna et al. 1995). Galstoff (1964) and Etim et al. (1991) observed that the ripe oyster and clam gonads, which may comprise as much 31%–41% of the total body weight, can have a great capacity to influence total tissue metal concentrations. Fowler and Oregioni (1976), Phillips (1976) and Boyden and Phillips (1981) studying *Mytilus galloprovincialis*, *M. edulis* and *Crassostrea gigas*, respectively, concluded that temporal variations in metal concentrations were mainly caused by changes in soft tissue weights of the bivalves according to the sexual cycle.

The freshwater clam, *Galatea paradoxa* (Born, 1778) (=*Egeria radiata* (Lamarck, 1804)) is stenotopic, being restricted in its mega-scale occurrence to a few large West African rivers namely, Volta River in Ghana, Nun and Cross Rivers in Nigeria, and Sanaga River in Cameroon (King and Udoidiong 1991). It is edible and widely distributed. An FAO species identification sheet shows that its range extends from the Gulf of Guinea to the Congo, but despite its wide range, the available literature indicates that it is exploited commercially in a few countries, like Ghana, Nigeria and Cameroon. In Ghana, the distribution of the clam is currently restricted to a very narrow stretch of the lower Volta River, between Agave-Afedume (15 km from the Volta estuary) and Ada-Foah (10 km from the estuary). It is interesting to note that the clam is not found in any other estuary or river in Ghana apart from the Volta. Throughout its geographic distribution, *G. paradoxa* supports a thriving artisanal clam fishery providing an affordable protein source and employment to the communities.

In recent years however, there has been a proliferation of rural metal fabrication industries along the Volta River basin and the estuary including Ada have been implicated as areas negatively impacted by the activities of these industries. Anecdotal information suggests that the basin might be receiving a considerable range of polluting effluents, particularly heavy metals, from these metal fabrication sources. The health risks associated with heavy metal poisoning in humans and the environment are of concern to environmentalists and government agencies locally and globally, and underscore the need for continuous research aimed at monitoring the levels of these pollutants, as well as putting in place measures to ameliorate the challenges of environmental pollution (Obirikorang et al. 2009).

The purpose of the present study was to provide baseline information on the concentrations of the Mn, Zn, Fe and Hg in sediment samples and the tissues of *G. paradoxa* and to evaluate the relationship between the sediment and tissue levels of the heavy metals at the Volta Estuary. Additionally, the relationship between gonadal development in *G. paradoxa* on the concentration of the four heavy metals (Mn, Zn, Fe and Hg) was evaluated.

Materials and Methods

The study was carried out at Ada on the Volta estuary, Ghana, over an 18-month period, from March 2008 to August 2009, and followed the procedures described in Obirikorang et al. (2010).

Clam samples were obtained from fishermen's catches at Ada, and transported in insulated chests to the laboratory within 24 h. The samples were processed for the calculation of gonadal condition indices (GCI), and subsequently for heavy metal analyses. In the laboratory, clam samples were cleansed to remove mud, and afterwards washed with double distilled water. They were then purged of ingested organic and inorganic particles before being analysed for heavy metal accumulation by keeping them in distilled water for a 24-h depuration. Thirty (30) sexually-mature clams with shell length over 55 mm were chosen each month for the analyses. A sterile stainless steel knife was used to dislodge and remove the soft tissue of each clam from the shell (Chiu et al. 2000). For the determination of the gonadal condition indices, the gonads which are associated with the mantle were carefully excised and weighed (gonad wet weight) on a Sartorius BP 210 S micro balance (0.0001 g), the rest of the tissue excluding the gonads was also weighed (tissue wet weight). The gonads and the rest of the tissue were oven-dried at 60°C for 48 h and weighed as gonad dry weight and tissue dry weight, respectively. The clam shells were air-dried and weighed (shell weight) to the nearest 0.1 g. Two gonadal indices of each specimen were computed as:

(a) Gonad dry weight (GDW)/shell weight (SW) × 100
(b) Gonad dry weight (GDW)/Total dry flesh weight ((TDFW) × 100

Each dry clam subsample was ground into fine powder using a porcelain pestle and mortar. Homogenized subsamples were stored in airtight, acid-washed (0.1MHCl) snap-top glass vials for heavy-metal analyses (United Kingdom Environmental Agency 2008).

Surface sediment samples (0–5 cm) were collected on a monthly interval for the 18-month period using an Ekman grab at the sampling location. The samples were collected according to the standard procedures described in USEPA's sediment sampling guide (USEPA 1994) and were kept in LDPE bottles pre-washed with 10% HC1 and stored in insulated iced chests at about 4°C for analyses in the laboratory. In the laboratory, the sediment subsamples of 500 g from each sampling location were placed in ceramic mortars for drying at 80°C for 48 h (Phillips and Yim 1981). The dried samples were then gently disaggregated, and 250 g of each sample stored in 250 mL acid-washed LDPE bottles for the heavy metal analyses (USEPA 1994).

Approximately 0.5 g of the homogenized clam and sediment samples were weighed into a 50 mL digestion tube and 1 mL of distilled water, 2.0 mL perchloric acid (HNO_3–$HClO_4$) (1:1 vv) and 5.0 mL sulphuric acid (H_2SO_4) were added. Each mixture was refluxed at 200°C for 30 min in a fume chamber. The completely digested subsamples were allowed to cool at room temperature, and the undigested portions filtered off through a Whatmann Glass Microfibre filter paper (GF/C) to obtain a clear solution and diluted to 50 mL in volumetric flasks with double distilled water (Jin et al. 1999; Otchere et al. 2003).

Concentrations of Mn, Zn and Fe were determined using a Buck Scientific Model VGP flame Atomic Absorption Spectrophotometer (AAS). All tissue analytical batches were accompanied by blanks at a minimum rate of one blank per 20 samples. Replicate analyses were conducted on all of the samples to evaluate the precision of the analytical techniques. The data were expressed as total concentration (µg/g dry weight (dw).

An Atomic Mercury Analyzer (Model HG 5000) equipped with a mercury lamp at a wavelength 253.7 was used for the determination of total mercury in the clam tissues and sediment samples. Responses were recorded on strip chart recorders as sharp peaks. The peak heights were used for computation of the total mercury concentrations in the clam and expressed as (µg/g dw). Total mercury concentrations were validated according to standard procedures described for the Mercury Analyzer Model HG 5000 to check for precision and accuracy.

The following physicochemical factors of the Volta River (pH, temperature, salinity, dissolved oxygen (DO), total dissolved solids (TDS), and conductivity) were measured at monthly intervals with a Hanna (HI 9828) multiparameter probe and at the same time as clam samples were collected.

Concentration of heavy metals (Mn, Zn, Fe and Hg) in sediments and clam tissues were measured as the mean ± SD. Linear Regression analyses were performed to ascertain the degree to which variations in the monthly gonadal indices were related to the levels of the four heavy metals in the whole tissue of *G. paradoxa*. All descriptive statistics and graphs were executed using the GraphPad Prism 5 software.

Results and Discussion

The measured physicochemical factors of the river were relatively constant over the 18-month sampling period with the exception of DO which fluctuated over the study period. The temporal trends for the physicochemical factors are shown in Fig. 1. pH ranged between 6.2 and 8.5 with a mean of 6.9 ± 0.5 over the sampling period. Temperature varied within a narrow range between 27.3 and 29.6°C with a mean of 28.6 ± 0.8°C. Salinity was relatively constant at 0.03 PSU throughout the study period. DO levels had a mean value of 4.2 ± 1.9 mg/L and ranged from a low of 1.5 mg/L in September 2008 to 8.8 mg/L in March 2008.

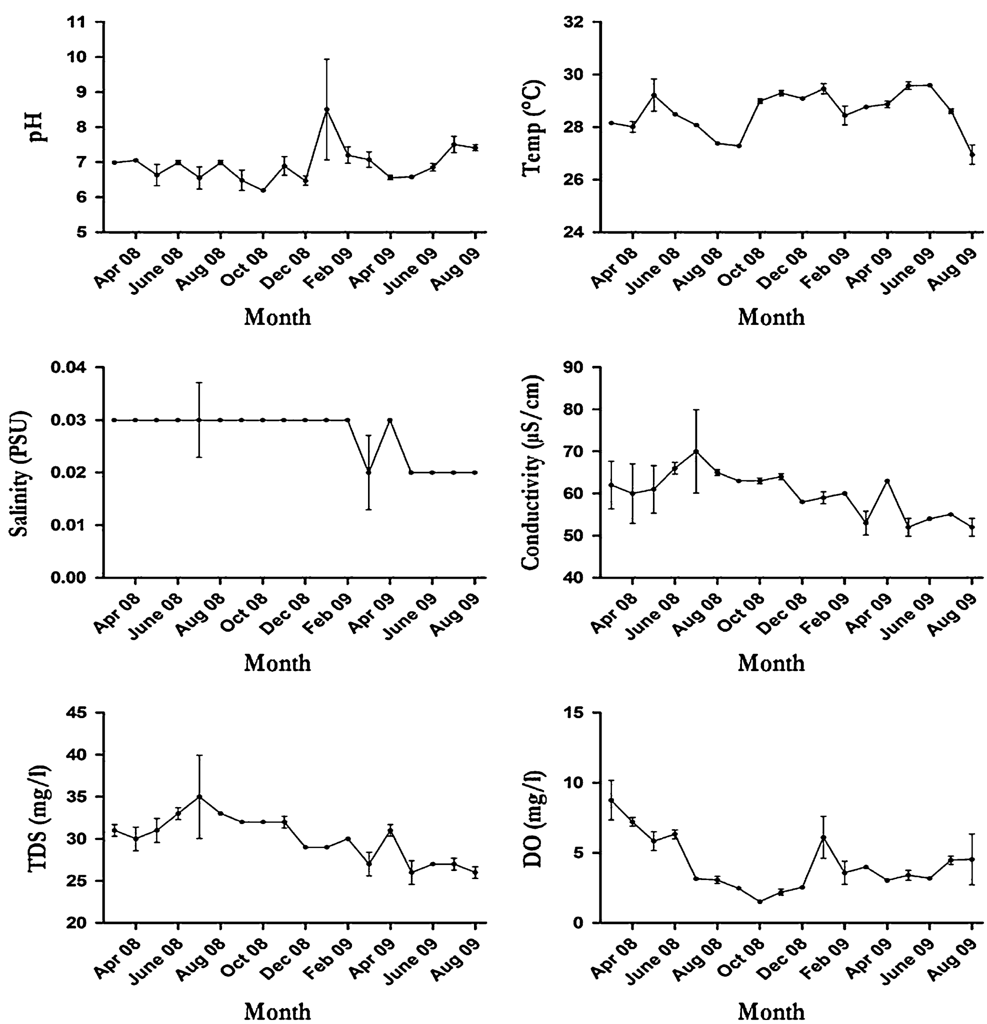

Fig. 1 Temporal trends in the physicochemical factors of the Volta River at Ada from March 2008 to August 2009

Table 1 Minimum and maximum heavy metal concentrations (μg/g dw) of Mn, Zn, Fe, and Hg in sediment samples compared to unpolluted sediment standards

	Mn	Zn	Zn	Hg
Min. conc. (μg/g dw)	147	ND	696	0.0069
Max. conc. (μg/g dw)	393	46	2,758	0.0140
Unpolluted sediment standard*	700	95	4,100	1

* GESAMP 1982

The values dropped steadily from March to October 2008 after which there was a progressive rise to the end of the sampling period, although there were periodic drops during certain months. The concentration of TDS was fairly constant with values ranging between 27 and 35 mg/L (mean 30.1 ± 2.7 mg/L) over the sampling period. Similarly, conductivity was relatively constant varying between 70 μS/cm in July 2008 and 52 μS/cm in May and August 2009.

Table 1 presents the minimum and maximum concentrations of Mn, Zn, Fe and Hg in sediment samples collected at Ada over the study period. The concentrations of the four metals were well below the unpolluted sediment standard (GESAMP 1982).

Temporal concentrations (mean ± SD) of Mn, Zn, Fe and Hg in the sediments and the whole tissue of *G. paradoxa* from Ada are presented in Fig. 2. Manganese (Mn) concentration in the whole soft tissue of the clams varied over a wide range from 49 to 867 μg/g. Mn levels were relatively constant around 100 μg/g for a greater part of the study period except in July and August 2008 when mean concentrations above 400 μg/g were recorded. The highest concentration of Zn in the tissue of the clams (59 μg/g) was recorded in December, 2008 with the lowest concentrations of 13 μg/g being recorded in March 2008. The results obtained for Fe revealed a maximum value of 484 μg/g in January 2009 and a minimum value of 79 μg/g in April, 2008. Hg concentrations ranged from 0.026 μg/g in January 2009 to 0.059 μg/g in September 2008.

Tissue levels of Zn and Hg were higher than the levels in the sediments while Fe concentration in the sediment was consistently higher than the levels in the clam tissue (Fig. 2). Apart from the period between July and August when tissue Mn levels were higher than the sediments, Mn levels in the sediment and tissues were relatively similar.

The physicochemical factors did not show any strong correlations with the sediment and tissue levels of the four heavy metals. No defined accumulation patterns were established for the metals (Mn, Zn, Fe and Hg). The *t* test also revealed no significant relationships ($p > 0.05$) as far as metal uptake from the sediments by the clams was concerned.

The risks associated with the consumption of *G. paradoxa* were ascertained by comparing the tissue concentration of the studied metals to the WHO safety reference standards for bivalves (WHO 2000). The analyses revealed that the concentration of the heavy metals in *G. paradoxa* tissues were within permissible limits for human consumption as far as Zn and Hg are concerned as shown in Table 2.

Figure 3 shows the variation in gonad dry weight over shell weight (GDW/SW) and gonad dry weight over total dry flesh weight (GDW/TDFW) during the study period

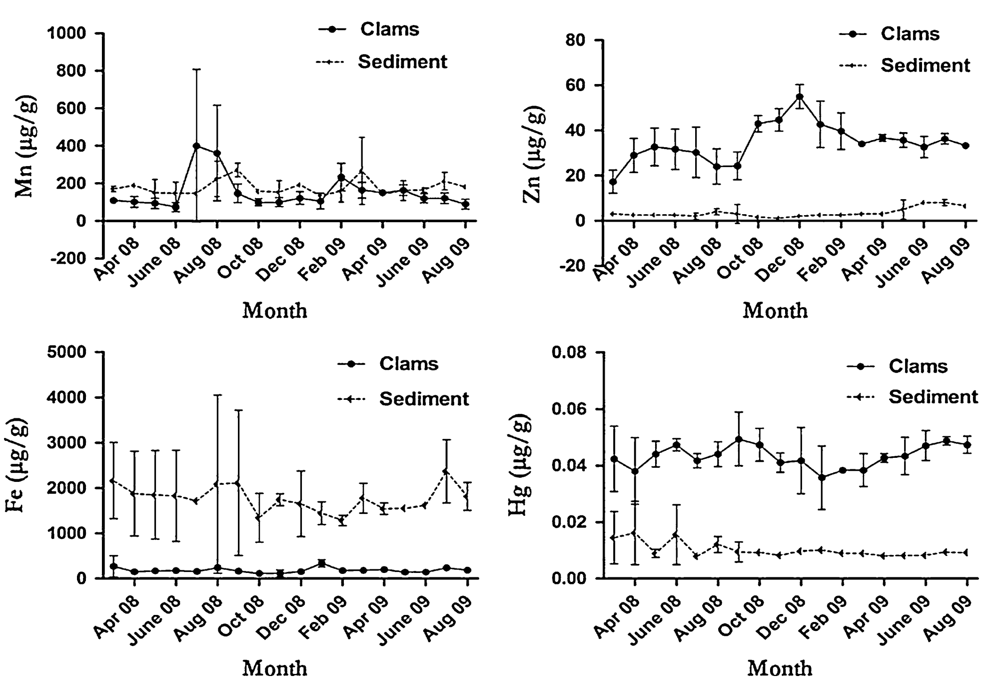

Fig. 2 Temporal variations in heavy metal (Mn, Zn, Fe, and Hg) concentrations in the tissues of *G. paradoxa* and sediment samples collected at Ada

Table 2 Minimum and maximum concentrations of Mn, Zn, Fe, and Hg (μg/g dw) in the whole soft tissue of *G. paradoxa* compared with WHO standards

	Mn	Zn	Fe	Hg
Min. Conc. (μg/g dw)	49	13	79	0.028
Max. Conc. (μg/g dw)	867	46	316	0.059
WHO standard (2000)	*	1,000	*	0.5

* No health-based guideline values were found

from March 2008 to August 2009. GDW/SW rose from 0.60 ± 0.05 in March 2008 to a peak of 1.72 ± 0.21 in August 2008. Afterwards, it fell to a minimum value of 0.50 ± 0.06 in January 2009. The pattern repeated itself in 2009 rising from 0.50 ± 0.28 in January to a peak value of 1.43 ± 0.86 in June. The variation of GDW/TDFW was similar to GDW/SW, the index rose from 19.11 ± 0.81 in March 2008 at the start of the study to a peak value of 34.17 ± 1.15 in August 2008. It declined steadily to a minimum value of 19.44 ± 1.31 in January 2009 and thereafter rose steadily to a peak value of 27.89 ± 1.15 in May 2009.

The reproductive cycle of *G. paradoxa* in the Volta River is annual with a single spawning event between June and October Adjei-Boateng and Wilson (in press). Gametogenesis in *G. Paradoxa* starts in December—January and progresses steadily to a peak in June-July when spawning begins until November when the animals are spent Adjei-Boateng and Wilson (in press). Mn exhibited a significant positive correlation ($p < 0.05$) between gonadal development and metal accumulation in the whole tissues of the clams. The peak Mn concentrations were usually recorded during the developing and mature stages of reproductive activity in *G. paradoxa*. This positive relationship between Mn concentration and gonadal development could be directly attributed to the accumulation of proteins and carbohydrates during spawning for gonad tissue production, energy storage and consumption (Latouche and Mix 1982; Páez-Osun et al. 1995). These proteins and carbohydrates have a high affinity for certain heavy metals, of which Mn could be part. According to Cunningham and Tripp (1975), if metals are accumulated in the gonad tissues, an appreciable loss might occur after spawning. This probably explains why the peak Mn concentrations coincided with the onset of the spawning period of *G. paradoxa* with sharp drops in concentrations soon after the spawning period. The behaviour of Mn is similar to the findings of Etim (1990) and Etim et al. (1991), whose studies on *G. paradoxa* in the Cross River, Nigeria, showed similar trends to the present study with peak metal concentrations coinciding with the spawning season of *G. paradoxa*. The Nigerian studies also revealed that spawning starts in June (when maximum mean dry tissue weight occurs), and is completed in October (minimum mean dry tissue weight). This observation corroborates the findings of the present study in which the spawning season of the Volta River stock of *G. paradoxa*, occurs at the peak of the rainy season in Ghana (June-July) and is completed just before the start of the dry season (November). Studies by Galstoff (1964) and Perera (2004) also reported positive correlations between gonadal development and Mn accumulation. According to Galstoff (1964), gonadal development and biochemical variations associated with reproduction are important in the accumulation of Mn than body size. Mean Hg concentrations also exhibited a slight positive relationship between gonadal development and metal accumulation, although this relationship was not significant ($p > 0.05$).

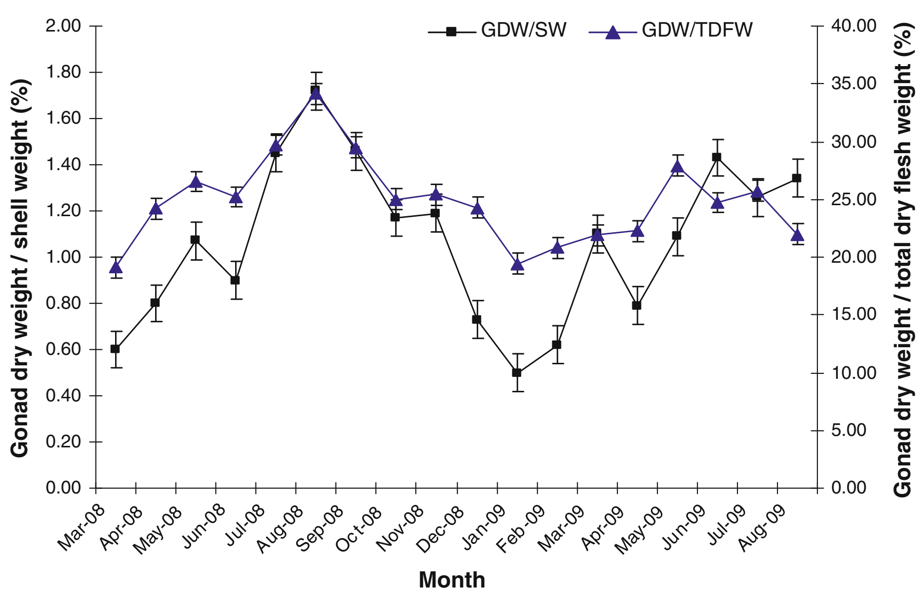

Fig. 3 Seasonal variation in gonad dry weight/shell weight and gonad dry weight/total dry flesh weight in *G. paradoxa* from the Volta estuary (mean ± SD) from March 2008 to August 2009

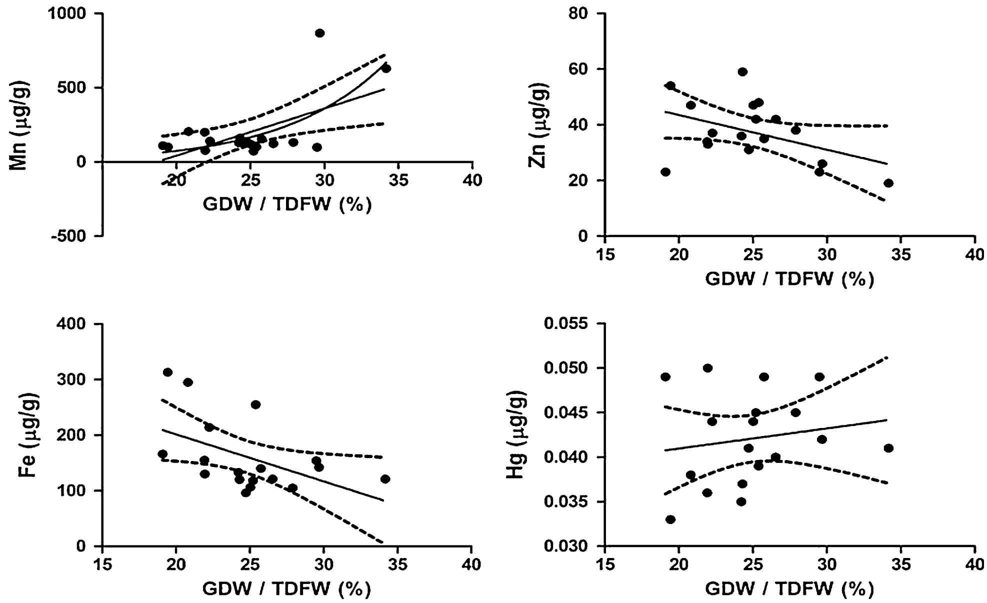

Fig. 4 Linear regression analyses between whole tissue metal concentrations and gonadal index

Table 3 Results of the linear regression analysis of gonadal index and whole tissue metal concentration for the studied metals

Metal	*n*	r^2	Slope	Y-intercept	F	*p value*
Mn	540	0.3190	13.45 ± 4.912	−181.7 ± 123.6	7.495	0.0146
Zn	540	0.05543	−0.5326 ± 0.5496	47.86 ± 13.83	0.9388	0.05543
Fe	540	0.1567	−6.544 ± 3.796	337.8 ± 95.51	2.972	0.1040
Hg	540	0.1730	0.000439 ± 0.000239	0.03231 ± 0.006033 3	3.346	0.0861

On the other hand, the period of gonadal ripening prior to spawning in *G. paradoxa* appeared to result in a decrease in the concentrations of Zn and Fe in the whole soft tissue. Zinc and Fe revealed a negative relationship between gonad development and metal accumulation, indicating that increase in gonadal condition indices of the clams resulted in lower concentrations of the two metals (Fig. 4). The negative relationship between gonadal development and metal accumulation as exhibited by Zn and Fe could be attributed to the dilution of the concentrations of these metals as a result of the rapid tissue growth that occurs prior to and just before the spawning period. Similar to the findings of this work, a study by Paez-Osuna et al. (1995) showed that some heavy metals including Zn are at their maximum concentrations during the resting or spent stages of gonad development in *Crassostrea iridescens.* A gradual drop in metal concentrations was observed with the development of gonads in the build-up to spawning. In another study by Zooragian (1980), it was observed that studied heavy metal concentrations with the exception of Mn exhibited a negative relationship with gonad tissue development, as was observed in this study (Table 3).

In conclusion, Mn exhibited a significant positive relationship ($p < 0.05$) between gonadal development and accumulation. Hg similarly exhibited a positive relationship although this was not significant. Zn and Fe revealed a trend of decreasing concentrations with gonadal development.

Although it is likely that the reproductive cycle of the clams plays an important role in the levels of metals in tissues, it will be ambiguous to interpret and conclude that the metal concentrations in *G. paradoxa* at the Volta estuary are solely affected by it, since the variability of heavy metal levels could also be caused by changes in the physiological conditions of the clams (Phelps et al. 1985) organic matter availability, (Pan and Wang 2004), sex (Sokolowski et al. 2003) and food acquisition capability (Saavedra et al. 2004).

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