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Trace Metals in the Brown Mussel *Perna perna* from the Coastal Waters Off Yemen (Gulf of Aden): How Concentrations Are Affected by Weight, Sex, and Seasonal Cycle

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Abstract. The effects of seasonal cycle, sex of individuals, and changes of soft tissues weight on accumulated trace metal concentrations (Cd, Cu, Fe, Mn, Pb, Zn) were examined in the brown mussel *Perna perna* collected monthly from a natural rocky habitat in the coastal waters off Yemen, the Gulf of Aden, for a period of ten months. Basic hydrological parameters were recorded simultaneously. All metals analyzed displayed seasonal fluctuations with different temporal patterns and variable amplitudes. Similar seasonal cycles were observed for Cu, Mn, and Pb with an increase in accumulated concentration during the rainy period (NE monsoon), and a decrease thereafter. The concentrations of Cu, Mn, and partially Pb appeared to be related to environmental changes, the concentration of Pb possibly also being related to changes in body weight. Accumulated concentrations of Cu and Mn thus seem to reflect actual metal bioavailability in the ecosystem quite efficiently. The tissue levels of Fe and Cd changed inversely to fluctuations in body weight with additional variation due to monsoon-related environmental changes. The behaviors of Fe and Cd are therefore driven by seasonally changing body weight with a considerable contribution of external factors including fluctuations in hydrological conditions and metal exposure. The Zn concentrations tended to increase gradually throughout most of the year regardless of its concentration in the environment. Zinc is considered to be mainly regulated by physiological mechanisms in the mussel, making its accumulated metal concentration independent to some degree of environmental levels. Significant differences in trace metal concentrations between sexes (in favour of females) might have resulted from more intense formation of reproductive tissues and metal accumulation in sexual products of females during the prespawning and spawning periods.

The use of cosmopolitan biomonitoring bivalves is widely recommended to define existing pollution levels (strictly pollutant bioavailabilities) and to provide a baseline against which future changes in such levels in the marine environment can be assessed. In particular the recommendations refer to tropical and subtropical areas where insufficient data are available for the assessment of aquatic contamination.

A number of national and international programs have been established over recent decades to identify efficient and accurate biomonitors of trace metal pollutant availabilities. Extensive surveys have been undertaken in Southeast Asia (Phillips 1985; Phillips and Rainbow 1988; Rainbow 1995), Australia (Klumpp and Burdon-Jones 1982), New Zealand (Nielsen and Nathan 1975), Thailand (Phillips and Muttarasin 1985), and lately in India (Lakshmanan and Nambisan 1989; Prakash *et al.* 1996). Biomonitoring studies in the waters off Yemen (the Gulf of Aden and the Red Sea) have lagged behind although this region was named by the International Marine Organisation in 1987 as an area exposed to a high danger of pollution. Some, background data on trace element pollution using surface sediments and some molluscs (the mussel *Perna perna,* the oyster *Saccostrea cucullata,* the clam *Pitar* sp., the gastropod *Turbo coronatus,* and the chiton *Acanthopleura haddoni*) in Aden Harbour and surrounding waters have been given by Ali (1997) and Szefer *et al.* (1997). No research, however, has been carried out so far on seasonal variation in metal accumulation or on biological factors affecting metal accumulation in mussel populations from the Gulf of Aden, northern Arabian Sea.

The accumulated metal concentration determined in a biomonitor is a time integrated measure of metal bioavailability in the natural habitat. This value can vary significantly following fluctuations in metal exposure and changes in hydrologic characteristics of the ecosystem (both directly affecting metal bioavailability and therefore rate of metal uptake) and temporal changes in the physiological state of the organism (affecting the accumulated concentration of the metal taken up). Therefore an understanding of the kinetics of metal accumulation, including physiological responses of the animals studied, can be a key feature in the evaluation of the suitability of a particular species as a biomonitor. Of the several processes that could affect metal flux rates in mussels, variations in environmental concentrations as well as the condition of an individual

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associated with its growth, gonadal development, spawning, sex, and age are commonly emphasized. Phillips (1980) reported that seasonal changes in zinc (Zn) concentrations in the mussel *Mytilus edulis,* for instance, are mainly due to dry weight fluctuations against a background of fairly stable metal content throughout a year. The effect of weight changes on metal concentrations has been also established for other marine and freshwater species of the family *Mytilidae* including *Mytilus galloprovincialis* (Majori *et al.* 1978), *Choromytilus meridionalis* (Orren *et al.* 1980), *Perna viridis* (Chu *et al.* 1990; Prakash and Rao 1993), *Perna indica* (Prabhudeva and Menon 1988), and *Velisunio ambiguus* (Jones and Walker 1979). Furthermore, many authors have suggested that tissue metal concentrations in mussels vary considerably according to reproductive stage and sex. On the other hand, Lobel and Wright (1982) provided evidence of the minor importance of gonadal development on metal levels which were obscured more by individual variability within populations.

The aim of this study was, therefore, to investigate seasonal changes in the soft tissue concentrations of cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), and Zn in the brown mussel *Perna perna* from tropical waters off Yemen (the Gulf of Aden) to provide a picture of the kinetics of trace metal accumulation. By relating these data to the temporal dynamics of body weight and the results of concurrent monitoring of basic hydrophysical parameters, the effects of variability in tissue dry weight and environmental conditions on metal concentrations in the mussels have been assessed. This aspect can be of importance for the optimisation of local aquaculture which produces mussels on a wide commercial scale. Intersex differences have also been determined here. The potential of *Perna perna* as a biomonitor of trace metal availability in Yemeni coastal waters is discussed with respect to selected aspects of the mussel's biology.

The brown mussel *Perna perna* belongs to the genus *Perna* that also contains the two species: *P. viridis* and *P. canaliculus*. No single taxonomic character can be used to discriminate unequivocally between these three species although the outer appearance of shells might sometimes be of a diagnostic value. Shells of adult *P. perna* are typically brown to red-maroon with irregular areas of light brown and green. Brilliant and blue green are dominant in *P. viridis* and light-colored zigzag markings are most common in young *P. canaliculus*. According to Siddall (1980), specific identification of living specimens of *Perna* species can be based on their distinct geographical distribution. *Perna perna* commonly appears in the coastal waters of eastern South America (Venezuela, Brazil, Uruguay), northwestern and southern Africa (Mozambique, Republic of South Africa), as well as in the Mediterranean Sea (Vakily 1989). The species has also been recorded in the Indian Ocean along the coasts of west India, Oman, and Yemen (Fowler *et al.* 1993). The mussels collected in the Hadramout district (Ash-shehr site), the Gulf of Aden, are not *P. viridis* and have been identified by analysis of shells by the Department of Zoology, The Natural History Museum, London as *Perna picta* (Born 1778), which may be synonymous with *Perna perna* (personal communication). Appreciating the confusion over specific identification in the genus *Perna,* the name *Perna perna* is used here for the Gulf of Aden mussels following the division of the genus by Siddall (1980) into three major species.

Study Site

The Gulf of Aden, as defined by the protocol relating to the Regional Convention on the Conservation of the Marine Environment and Coastal Areas of the Red Sea and the Gulf of Aden, extends from the southern entrance to the Red Sea (through the narrow Strait of Bab el Mandeb) westwards between the Arabian Peninsula and the Somali Peninsula, and then opens into the northern Arabian Sea (PERSGA 1981). The approximate surface area is 220×10^3 km^2 of which the continental shelf covers a strip 40–50 km wide. The Yemeni shore is located along the northern edge of the gulf. Its shape varies according to the geological history of each region and the dominant features are repeated rocky headlands between which series of cuspate beaches have formed. The influence of the major wadi systems (dry river beds in a desert) is apparent, fluvial deposits being accumulated on the coast below them.

The hydrography and ecological structure of the Gulf of Aden are mainly affected by the reversal of the wind regime and water exchange with the Red Sea. The littoral zone off Yemen constitutes a relatively well-mixed and well-oxygenated, warm water body with fairly high salinity (salinity range 33.7–37.0) and an average tidal amplitude of 1.0 m. The biodiversity of this region strongly depends on the seasonal monsoons that occur twice a year. The stronger and steady southwest (SW) monsoon, which is usually restricted to the late summer (July to September), is the major force involved in generating upwelling that brings cold nutrient-rich water from the deep ocean and results in a marked rise in biological productivity. Chlorophyll *a* concentration in surface layer can then reach 1.0 mg C m^{-3} h^{-1}, a value typical for areas of the highest oceanic primary production, such as the Peruvian upwelling region (Smith 1984). The most conspicuous ecological effect of the upwelling is, however, the appearance of vast growths of subtidal and intertidal algal assemblages covering the exposed platforms and rocks in high densities. By contrast, during the northeast (NE) monsoon (October to March) primary production and algal populations decline in consequence of depressed nutrient loads, decreased water temperature and torrential rains.

The waters off Yemen are now threatened by several kinds of pollution from passing ships and local shore facilities. Due to the special position of the gulf in the main pathway of oil transportation from the Middle East and Asia, as well as the development of oil industry in the area, considerable attention has lately been paid to oil pollution.

Ash-shehr, where mussels were collected, is located on the southern coast of Yemen, about 550 km north of the Aden district, which is the most industrialized region of the country. The sampling site has been selected with the intention that it represents an area relatively unaffected anthropogenically, subjected only to minor effluents of fishery refrigerators discharged to the sea locally.

Materials and Methods

Mussels Perna perna

Samples were collected monthly from the intertidal zone at one site Ash-shehr on the west coast of the Gulf of Aden, Yemen (Figure 1)

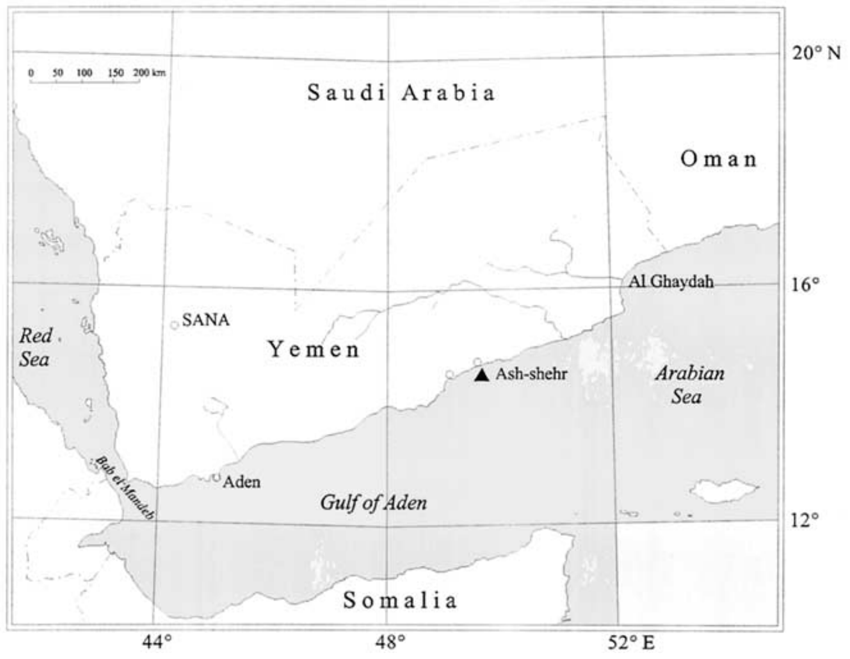

Fig. 1. Location of sampling site (▲).

between July 1997 and April 1998. The sampling location was based upon previous surveys of mussels to represent wild populations of mussels inhabitating rocky shores in an unpolluted area. Ash-shehr is situated in an area far from obvious sources of metal pollution and is considered to be one of the main centers of fisheries along the coast. Living specimens of *P. perna* were transported immediately to the laboratory in clean polyethylene bags, and were kept moist and cool during transport. The mussels were allowed to depurate the contents of the alimentary canals for 24 h in filtered surface seawater of ambient temperature. Shells were cleaned of debris and seaweeds, and the byssus was discarded. Thereafter, the samples were sorted into two size classes according to shell length: size class A (30–49 mm) and size class B (50–69 mm). For each month three analytical groups were prepared, in which seven individuals were pooled for each size class to obtain mean metal concentration in the samples (exceptions were samples collected in October and November, when only one analytical group was prepared for the size class A). In July, August, and September, 42 additional individuals were collected for each month, sexed and grouped into two sex groups consisting of three replicates, seven individuals each, to assess the importance of sex in influencing metal concentrations (Table 1). In this case, special care was taken to separate mussels of similar size (shell lengths were within 50–59 mm), as size is known to sometimes affect metal levels in mussels (Phillips 1980). Velez and Epifanio (1981) reported that for the family *Mytilidae* the beginning of the tropical summer (July to September) constitutes a peak of the spawning period during which gonads of mature organisms are fully developed and ripe, making the sex of a specimen easy to recognize.

All instruments used for trace metal analysis were acid precleaned (1M HNO_3) for 24 h to avoid contamination in any part of the procedures. The wet soft tissue of each individual was removed using disposable polyethylene tools, air dried at 55°C to a constant weight, and weighed to determine the average soft tissue dry weight of the mussels. After homogenization in a ceramic mortar, three replicate 0.3-g subsamples were digested with 3 mL concentrated HNO_3 (Suprapur Merck) in Teflon vials in a microwave digestion system and made up to 5 mL with Milli-Q water (Millipore). Metal concentrations were determined by atomic absorption spectrophotometry using a Shimadzu AA-6501 instrument in flame mode with deuterium-background correction for all elements except Cu, which was measured without background correction. Data quality control was provided by a separate comparative study of a standard reference material (NRC, Canada TORT-1, lobster hepatopancreas) and procedural blanks, which were analyzed according to the same procedure every six samples. The agreement between the results for the reference biological material and the NRC certified values was satisfactory. Recovery (total certified concentrations of a metal versus total metal concentration in this study) and precision, which was calculated on the basis of the coefficient of variation, varied among metals analyzed and were $>93.6\%$ and $<10.7\%$, respectively (Table 2). The limit of detection (LOD) was calculated as three times the standard deviation of the blank.

Hydrological Parameters

Basic hydrological parameters of surface coastal waters—temperature, salinity, concentration of dissolved oxygen, contents of suspended particulate matter (SPM), chlorophyll *a*, and particulate organic matter (POM)—were recorded simultaneously at the same sampling site as the molluscs. Temperature, salinity, and the concentration of dissolved oxygen were measured directly in the field with a WTW Universal Pocket Meter MultiLine P4 while SPM, chlorophyll *a* and POM were determined in laboratory according to standard methods recommended for marine waters (Strickland and Parsons 1968; Kramer *et al.* 1994).

Statistical Analysis

Untransformed data were included in all statistical models followed by analyses of normality (Kolmogorov-Smirnov and a test of goodness of fit) and homogeneity of variances as prerequisites to the parametric approach. The significance of individual differences between two data groups was checked by a *t*-test and one-way ANOVA, and comparison of two time-series was performed with a correlation analysis. The relationship between pairs of variables was estimated with a regression

Table 1. Biometric data for two size classes (A: 30–49 mm and B: 50–69 mm; for soft tissue dry weight see Figure 5) and sex groups (determined in mussels sampled from July to September; size restricted to 50–59 mm) of *Perna perna* collected in Ash-shehr on the coast of the Gulf of Aden, Yemen (data are expressed as mean and standard deviation per sample; ♂-male, ♀-female)

Month	Size class	n^a	Shell length [mm]	Size class	Sex	n^b	Shell length [mm]	Soft tissue dry weight [g]
July	A			B	♂	3 (7)	56.7 ± 2.8	0.86 ± 0.43
	B	3 (7)	58.3 ± 4.4	B	♀	3 (7)	56.4 ± 3.0	0.84 ± 0.38
Aug	A	3 (7)	44.1 ± 4.5	B	♂	3 (7)	54.2 ± 3.1	0.82 ± 0.63
	B	3 (7)	56.4 ± 5.6	B	♀	3 (7)	53.0 ± 2.3	0.69 ± 0.49
Sep	A	3 (7)	43.7 ± 4.5	B	♂	3 (7)	54.9 ± 2.8	0.56 ± 0.15
	B	3 (7)	56.8 ± 3.4	B	♀	3 (7)	55.7 ± 2.1	0.65 ± 0.20
Oct	A	1 (7)	44.6 ± 3.7					
	B	3 (7)	61.6 ± 5.6					
Nov	A	1 (7)	42.8 ± 5.8					
	B	3 (7)	57.7 ± 5.5					
Dec	A	3 (7)	47.4 ± 0.9					
	B	3 (7)	56.6 ± 3.5					
Jan	A	3 (7)	44.0 ± 4.8					
	B	3 (7)	53.8 ± 2.3					
Feb	A	3 (7)	45.4 ± 3.7					
	B	3 (7)	55.2 ± 5.5					
March	A	3 (7)	47.2 ± 1.9					
	B	3 (7)	52.7 ± 2.5					
April	A	3 (7)	42.2 ± 3.6					
	B	3 (7)	53.2 ± 3.9					

[a] Number of pooled samples and number of individuals per sample in parentheses.

[b] Number of pooled individuals per sample.

Table 2. Comparisons of mean metal concentrations in the biological reference material (NRC, Canada TORT-1, lobster hepatopancreas) [μg g^{-1} DW] with the results of this study along with data on the blanks and the limit of detection (LOD)

Metal	Cd	Cu	Fe	Mn	Pb	Zn
NRC certified value	26.7 ± 0.6	106 ± 10.0	105 ± 13.0	13.6 ± 1.2	0.35 ± 0.13	180 ± 6.0
this study	25.0 ± 0.7	103.1 ± 2.0	113.2 ± 12.1	14.5 ± 0.3	0.33 ± 0.10	175.1 ± 3.9
recovery [%]	93.6	97.3	107.8	106.6	94.3	97.3
detection limit	0.013	0.041	0.467	0.034	0.094	0.110
precision [%]	2.7	1.9	10.7	1.9	3.0	2.6
blank*	0.63	0.66	0.84	0.65	0.01	2.20

* Expressed as a mean value, $n = 3$ [μg dm^{-3}].

analysis and comparison of the slopes of the regression lines between two groups of related pairs was made with a test of equality of slopes in ANCOVA. To determine the effects of two independent variables (month and sex) for each metal, a two-way ANOVA test with Bonferroni correction at a critical probability of $\alpha' = \alpha/6$ was employed. More general estimates explaining variation in metal concentration and metal content in terms of environmental parameters was obtained by Multiple Regression Analysis. The level of significance for all tests except those with Bonferroni correction was set as $p < 0.05$. Analyses were performed using the PC professional software STATISTICA, Statsoft. Inc., USA (Sokal and Rohlf 1995).

Results

Hydrological Conditions

Hydrological conditions of surface water in the coastal zone at Ash-shehr demonstrated considerable seasonal variations (Figure 2), the most pronounced for chlorophyll *a* concentration, suspended particulate matter (SPM), and its organic component (POM). On the basis of the temporal distribution patterns of the environmental variables measured, two main periods of different hydrographical characteristics can be distinguished: late summer through early autumn (July through September/October) and autumn through spring (November through March), reflecting the effects of the two seasonal monsoons. During the late summer/early autumn months (corresponding to the SW monsoon) surface waters were relatively cool (down to 24.4°C in July); of high salinity, with a maximum value in October (36.8); and well-oxygenated, up to 8.7 mg dm^{-3} O_2 in July; features appearing typically after upwelling. This was accompanied by enhanced primary production in the water column as indicated by the elevated chlorophyll *a* concentration, POM, and SPM (up to 3.9 mg dm^{-3}, 4.7 mg dm^{-3}, and 42.3 mg dm^{-3} in September, respectively). Fairly distinct water conditions occurred in winter and spring, most likely as a result

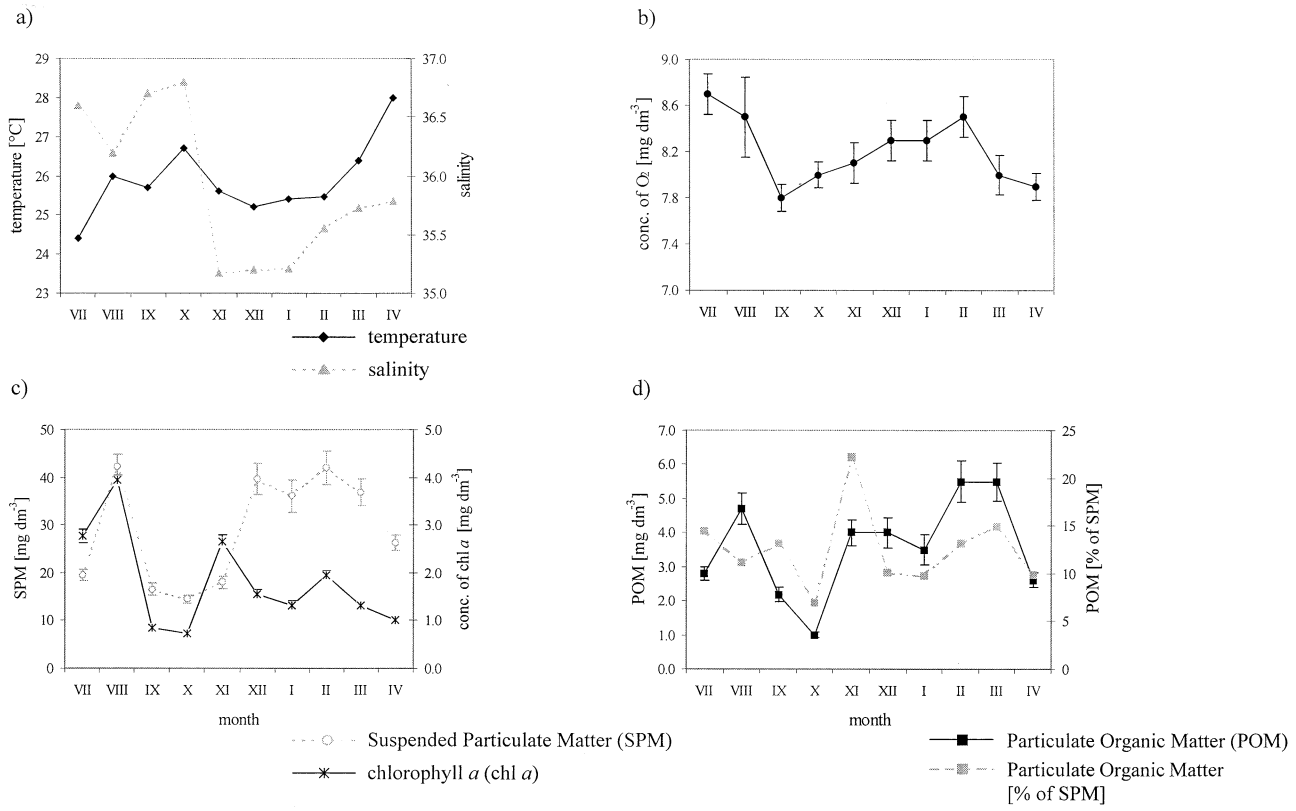

Fig. 2. Seasonal variations of temperature and salinity (**a**), concentration of dissolved oxygen (**b**), chlorophyll *a* and Suspended Particulate Matter (**c**), and Particulate Organic Matter (**d**) in surface coastal waters off Yemen (Gulf of Aden). Data are presented as mean ± standard error ($n = 3$).

of the NE monsoon bringing torrential rains and usually weak variable winds. Water temperature declined to 25.2°C in December and salinity decreased and remained low over November through January (35.2); both increased gradually in spring, reaching the highest values in April (28.0°C and 35.8, respectively). The lowest concentration of dissolved oxygen was noticed in September (7.8 mg dm^{-3}), followed by a slight increase until February and a subsequent decrease thereafter. Similar temporal distributions were observed for chlorophyll *a* and POM, suggesting that planktonic production accounted primarily for organic particles in the water column and that the contribution of terrestrial input had limited importance. The effect of biological productivity on the organic components of suspended matter was particularly clear during the SW monsoon (July through September) and in the two following months (October through November), when POM contained on average 71.9% of chlorophll *a,* as compared to 35.0% in the remaining period. Chlorophyll *a* concentration and POM declined from September to their minimal levels in October (0.72 mg dm^{-3} and 1.0 mg dm^{-3}, respectively), then rose markedly in November and February, to decrease again in April (Figure 2). An approximately similar temporal pattern was also observed for SPM with two main periods of elevated values in late summer (maximal concentration 42.3 mg dm^{-3} in September) and winter (December through February), separated by an autumn minimum (14.5 mg dm^{-3}).

Metals in Mussels

Monthly mean concentrations of each metal in the soft tissue of *P. perna* are presented graphically (Figure 3). Concentrations of all elements examined showed clear seasonal fluctuations in both size classes (one-way ANOVA, $p < 0.05$). The amplitude of annual variations was expressed as a seasonal factor, which corresponds for each metal to the ratio of the highest concentration to lowest concentration (Bordin *et al.* 1992). The highest values of this factor were recorded for Mn and Cd, a moderate value for Fe, and rather low values for Pb, Zn, and Cu. It is noteworthy that for Mn, Cd, Fe, and Pb, the seasonal factor was substantially greater in smaller mussels (size class A) and similar in both size classes for Zn and Cu (Table 3).

Similar temporal patterns were observed for Mn, Pb, and Cu, with a winter increase (December through January), followed by an additional peak for Mn and Cu in October, and an early spring decrease (February through March). In contrast, throughout much of the year, Zn concentrations slightly increased reaching a maximum in March, followed by a decrease in April. Elevated concentrations of Fe and Cd occurred in autumn i.e. in October and November, respectively with lower but variable levels over the remaining months (Figure 3). Correlation analyses showed no statistically significant differences between any two time-series for concentrations of Cu, Fe, Cd, Mn, and Zn in mussels of the two separate size classes

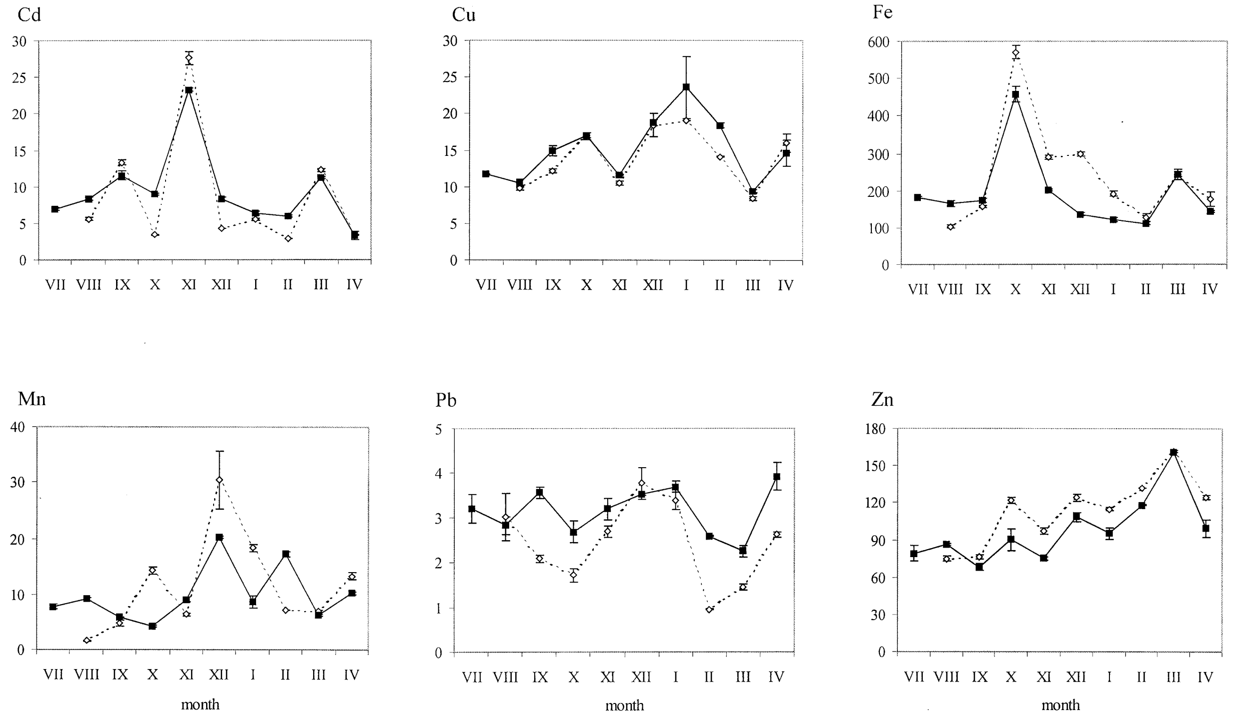

Fig. 3. Seasonal changes in soft tissue metal concentrations [μg g^{-1} DW] in two size classes of *Perna perna* (mean ± st. error; $n = 3$).

Table 3. Seasonal factors expressed as the ratio of the highest metal concentration to the lowest metal concentration [μg g^{-1} DW/μg g^{-1} DW] for each size class of *Perna perna*

Size class	Cd	Cu	Fe	Mn	Pb	Zn
A	9.34	2.27	5.52	19.39	3.92	2.16
B	6.96	2.43	4.08	3.49	1.75	2.49
mean	8.15	2.34	4.81	11.44	2.84	2.33

($p < 0.05$). Furthermore, metal content (μg ind^{-1}), as opposed to metal concentration (μg g^{-1}), was also calculated for both size classes in each month (Figure 4) in order to assess the effect of environmental exposure of an element on its tissue accumulation (Cossa *et al.* 1980). No statistically significant differences between any two time-series for contents of Cu, Cd, Mn, and Zn in mussels of two separate size classes were apparent (correlation analysis, $p < 0.05$), but the effect of time (months) was clear for all metals and in both size classes (one-way ANOVA, $p < 0.05$). Comparative analyses of the curves illustrating seasonal variations in metal concentrations and seasonal variations in metal content (separately for each size class and for two classes combined) revealed good agreement between the two corresponding curves for all elements, except Zn in the size class 30–49 mm and in two classes combined (correlation analysis, $p < 0.05$). The highest Zn content over the period analyzed occurred in both size classes in December and the lowest in November, while Zn concentration reached a maximum in March.

Data on the soft tissue dry weight of the brown mussels are shown in Figure 5. Temporal changes of dry weight were strongly affected by both season and size (two-way ANOVA, $p < 0.001$). The growth cycle of *P. perna* was characterised by a gradual loss of weight during the summer and autumn (July through November), a rapid increase in December, and a subsequent decrease in tissue weight in early spring (February through March). Temporal variation in dry weight was more distinct for smaller mussels (seasonal factor, as indicated by the ratio of the highest weight to the lowest of 2.96) than for bigger ones (1.80). No statistical differences, however, between the two series were apparent (correlation analysis, $p < 0.05$). Since it has often been suggested that variations in soft tissue dry weight lead to changes in metal level in mussel's body, the regression analysis of tissue metal concentration and tissue metal content versus tissue dry weight was computed for each element. Due to possible differences in the slopes between size classes, as suggested by the effect of size on soft tissue dry weight, regression analysis was performed separately for each size class and the slopes of the regression lines were compared (test of equality of slopes in ANCOVA, $p < 0.05$). The results are shown in Figures 6 and 7 and in Table 4. Positively sloped relationships were noted for the concentrations of Pb, Cu, and Mn, of which only the Pb correlation was significant (for two size classes combined, $p < 0.05$). Zn, Fe, and Cd exhibited negatively sloped relationships. No significant differences between separate regressions of metal concentration on dry weight for any size class in slope were recorded (a test of equality of slopes, $p < 0.05$). The contents of all metals appeared to be positively related to the average body weight (for two size classes combined), suggesting dependence on individual size. Significant correlations were found for Cu, Mn, Pb, and Zn, the highest correlation coefficients being apparent for Pb and Cu ($R = 0.88$ and $R = 0.71$, both $p < 0.001$,

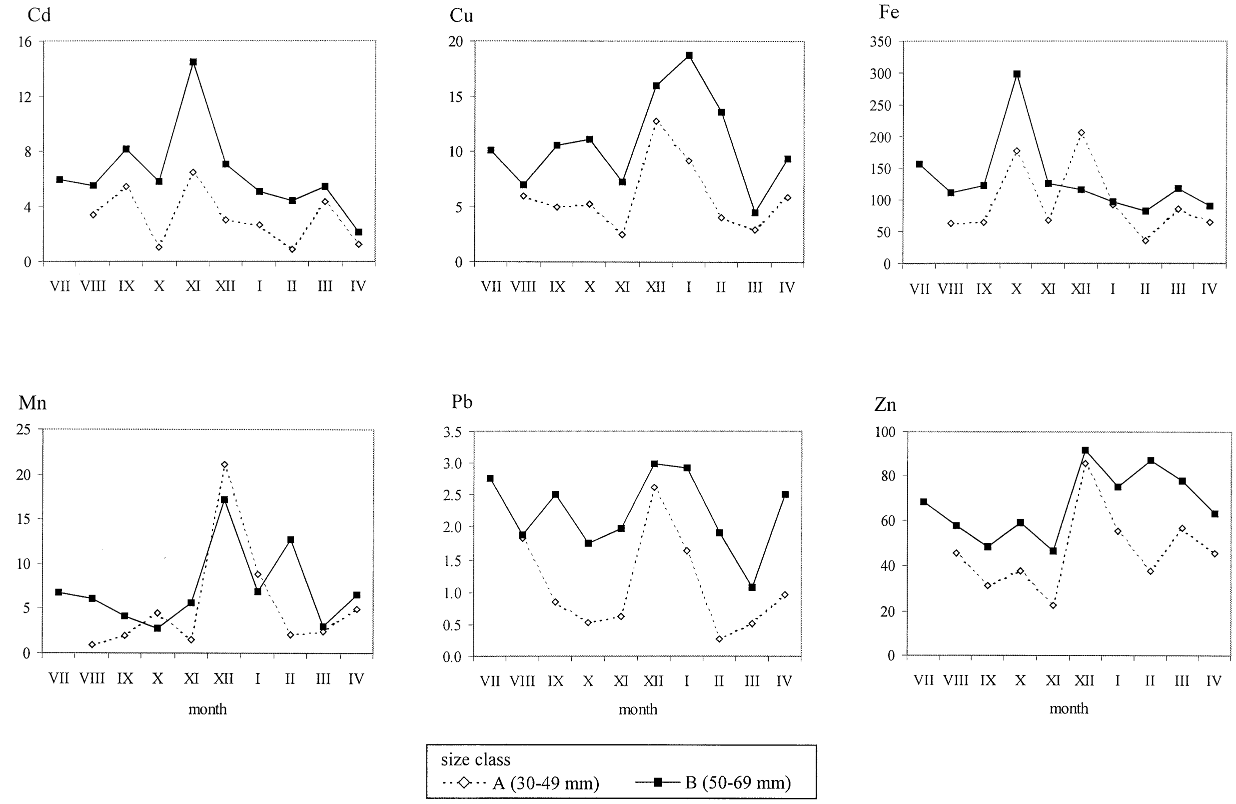

Fig. 4. Seasonal changes in soft tissue metal content [μg ind^{-1}] in two size classes of *Perna perna*.

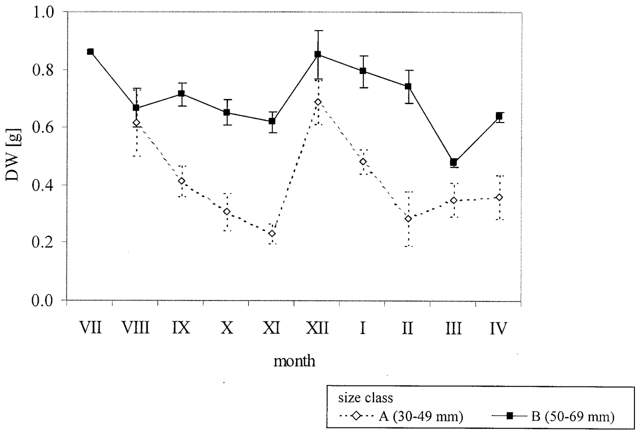

Fig. 5. Seasonal changes in soft tissue dry weight of *Perna perna* in two size classes (mean ± st.error; $n = 7$).

respectively), and the lowest for Zn and Mn ($R = 0.55$, $p < 0.001$ and $R = 0.29$, $p < 0.02$, respectively). The regression slopes for the separate size classes did not differ for any element, except Fe. It is noteworthy that the slopes of the regressions between Cd content and dry weight were negative when considering each size class separately and positive when the two classes were analyzed together (Table 4). Results of Multiple Regression Analysis performed on metal concentration and metal content in the soft tissue of *P. perna* and environmental data show that the effects of particular hydrological parameters on metal level in the mussels were apparent only for the smaller size class (A: 30–49 mm), and varied considerably between metals (Table 5). Salinity, chlorophyll *a*, and POM appeared to affect Cu; temperature and dissolved oxygen were negatively correlated with Cd content; while Pb content was influenced the most by POM and SPM. No significant multiple regression correlations were found for Fe, Mn, or Zn.

In order to assess the possible differences in soft tissue metal concentration between sexes, three separate collections of mussels (63 males and 63 females in total) were made from clumps in July, August, and September and pooled into two main sex groups. Results are presented in Figure 8. For all metals, except Fe, average concentrations were greater in females than in males, although an intersex difference was statistically proved only for Mn (*t*-test, $p < 0.05$). Lack of significant differences between males and females can be attributed to temporal fluctuations related presumably to changes in soft tissue dry weight as suggested by the data in Table 1. Significant effects of time (month) and sex and interaction effects on metal concentration were observed for all elements, except Mn (a two-way ANOVA test with Bonferroni correction at a critical probability of $\alpha' = \alpha/6$), for which the effect of time was not apparent. This may explain the significance of differences between sex groups noticed for Mn. In the case of Fe, a slightly higher concentration was recorded in males than in females.

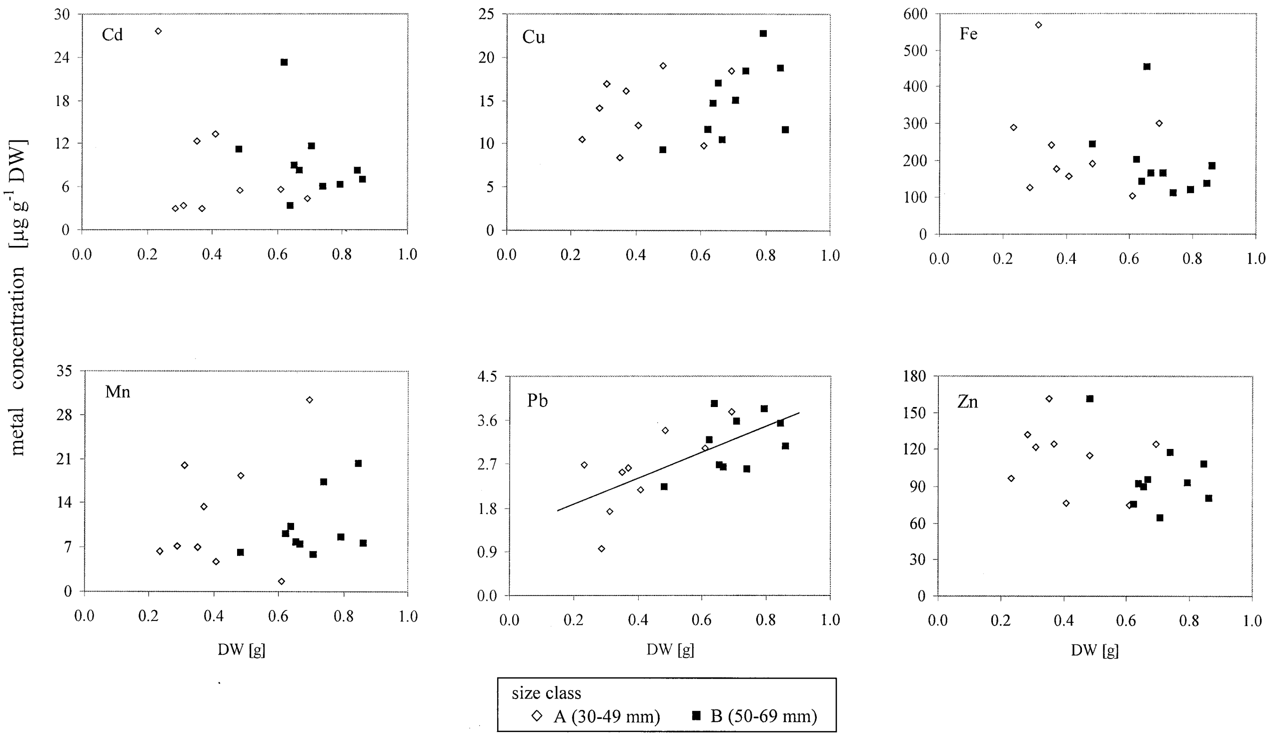

Fig. 6. Relationships between soft tissue metal concentrations and tissue dry weight of *Perna perna.*

Discussion

Environmental Conditions

Environmental conditions observed at the Ash-shehr site, the Hadramout district, during the study period reflected in general the overall climatic and hydrological situation of the northern part the Gulf of Aden. The area is subjected to the effects of two global phenomena, namely the southwest and northeast monsoons with two transition periods that control the hydrography and ecological characteristics of the tropical zone. The impact of the monsoon seasons was also clearly discernable on the temporal variations of the environmental variables measured. During the summer/autumn months (July through October), a marked increase in salinity of surface waters was observed, coinciding with a decrease in the concentration of dissolved oxygen, most probably as a result of upwelling induced by the strong offcoast winds of the SW monsoon. Enrichment in nutrients of the euphotic layer caused by the upwelled waters and increasing temperature stimulated primary production of phytoplankton resulting in elevated concentrations of chlorophyll *a* and SPM (Figure 2). The contribution of chlorophyll *a* on SPM and POM in this period reached maximal of 14.7% and 99.0%, respectively. A considerable change in hydrological conditions at the sampling site (a drop in salinity and temperature and an increase of SPM, among others) occurred in November through December, indicating the beginning of the NE monsoon season that brings torrential rains. Although the precipitation lasts usually only a short time (USOCD 1982), the inflow of fresh water to the coastal zone is extended for the following weeks due to water input from the system of small wadies. Seasonally formed streams and rivers discharge into the water from wide areas inland and often carry great quantities of terrestrial materials which sediment in the coastal zone. The effect of elevated input of terrigenic nonorganic deposits was apparent at Ash-shehr in November through April, with an increased load of SPM (up to 42.0 mg dm^{-3} in February) and a lowered concentration of chlorophyll *a,* in particular between December and April (Figure 2).

Seasonal Fluctuations of Metals and the Effects of Weight

Seasonal fluctuations of soft tissue metal concentrations in mussels may be driven by various environmental and biological factors. Of the processes affecting metal dynamics in mussels, variability in physicochemical conditions of the water and sediments (temperature, salinity, pH, suspended particulate matter content, etc.) related to changes in the concentrations of biologically available metals, and temporal variability in the physiological state of an organism (related to reproductive and/or feeding cycle) are considered important (Bordin *et al.* 1992). Discrimination between their respective influences is difficult to demonstrate clearly, due to complex interactions of different variables and the specific metabolic strategies of each species. The overall pattern of metal concentration variations can therefore vary between zoogeographical zones and between organisms, and is largely dependent upon local characteristics of the ecosystem. The degree by which a given factor can explain changes in metal concentrations in mussels differs also with elements for which geochemical behavior and metabolic pathways of accumulation are often distinct.

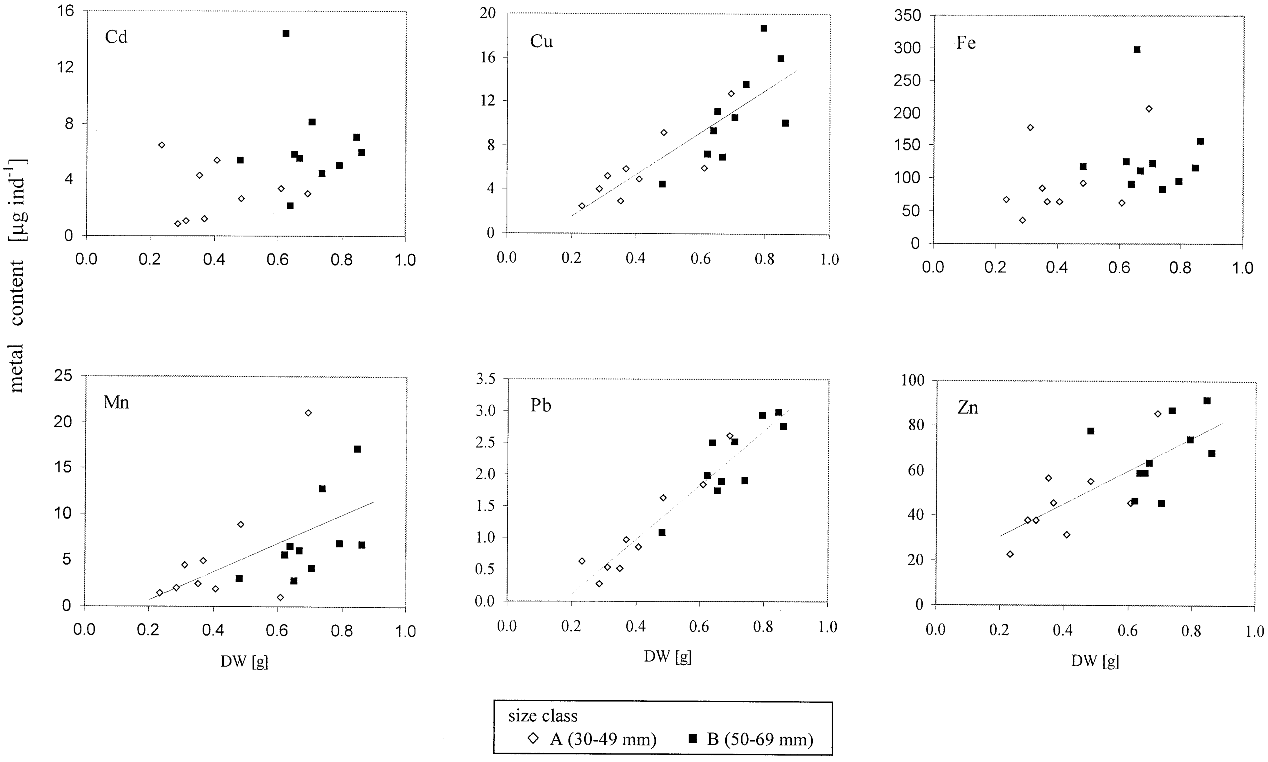

Fig. 7. Relationships between soft tissue metal content and tissue dry weight of *Perna perna.*

Table 4. Determination coefficients (R^2), levels of significance (p-levels), and the slopes of the linear regression between metal concentration and content and soft tissue dry weight calculated separately for each size class of *Perna perna* (A and B) and for two classes combined (A + B)

	Cd		Cu		Fe		Mn		Pb		Zn	
	R^2/p	slope	R^2/p	slope	R^2/p	slope	R^2/p	slope	R^2/p	slope	R^2/p	slope
Metal concentration												
A	0.18/0.257	−22.4	0.08/0.461	7.3	0.05/0.560	−209	0.17/0.269	25.1	0.53/0.27	4.0	0.06/0.537	−43.1
B	0.13/0.309	−17.0	0.10/0.096	20.7	0.15/0.275	−333	0.22/0.176	19.5	0.21/0.185	2.3	0.20/0.199	−104
A + B	0.05/0.364	−7.5	0.14/0.120	7.6	0.11/0.162	−205	0.03/0.488	6.2	0.46/0.001	2.6	0.19/0.071	−60.4
Metal content												
A	0.01/0.906	−0.59	0.69/0.006	17.6	0.19/0.235	167	0.45/0.049	28.0	0.90/0.001	4.8	0.59/0.015	92.6
B	0.01/0.823	−2.29	0.56/0.012	28.1	0.01/0.842	−39*	0.38/0.057	24.1	0.73/0.002	4.5	0.12/0.333	45.2
A + B	0.13/0.124	5.9	0.71/0.001	19.0	0.12/0.147	109	0.29/0.017	14.8	0.88/0.001	4.2	0.55/0.001	73.8

* Indicates statistical differences between separate regressions of metal concentration and metal content on dry weight for any size class in slope (test of equality of slopes in ANCOVA, $p < 0.05$).

Taking into account these divergences, regional, and species-orientated investigations are needed to define the mechanisms controling soft tissue metal accumulation in mussels. Evaluation of the contribution of the potential factors in spatiotemporal fluctuations may be essential for establishing environmental monitoring programs (Rainbow 1995).

The present study extends such research to comparisons of the mussel *P. perna,* collected from an area for which toxicological and biological data are very rare.

All metals examined displayed distinct seasonal variations, the most pronounced being for Mn and Cd as shown by the values of the seasonal factor. To assess the effect of environmental exposure of an element on its tissue accumulation, metal content (µg ind^{-1}) was also calculated for both size classes in each month (Cossa *et al.* 1980). This approach is commonly used to discriminate between the respective influences of environmental and biological factors on metal concentration fluctuations in bivalve soft tissues (Bordin *et al.* 1992). Another approach to the discrimination of the relative effects of external and internal factors on metal accumulation in mussels includes normalization on the condition index (Lobel *et al.* 1991), shell length (Cossa and Rondeau 1985), and shell weight, or the use of the index "metal content/shell weight" (Fisher 1983). However, in this study, owing to the lack of data on weight and morphology of the shell, the effects of different variables on metal accumulation in *P. perna* were

Table 5. Results of Multiple Regression Analysis of metal concentration and metal content in the size class A (30–49 mm) of *Perna perna,* and the environmental data

	Cu		Cd		Pb	
	Conc.	Content	Conc.	Content	Conc.	Content
T				**/–		
S	*/–					
O_2				***/–		
chl *a*	*/–					
POM	*/–	*/–				*/–
SPM						*/+

T, water temperature; S, salinity; O_2, oxygen concentration; chl *a,* chlorophyll *a* concentration; POM, particulate organic matter; SPM, suspended organic matter; * $\alpha < 0.05$; ** $\alpha < 0.01$; *** $\alpha < 0.001$; – negative relation; + positive relation; open fields are nonsignificant relations. No significant relations were found for Fe, Mn, and Zn in the size class A, and for all metals in the size class B (50–69 mm).

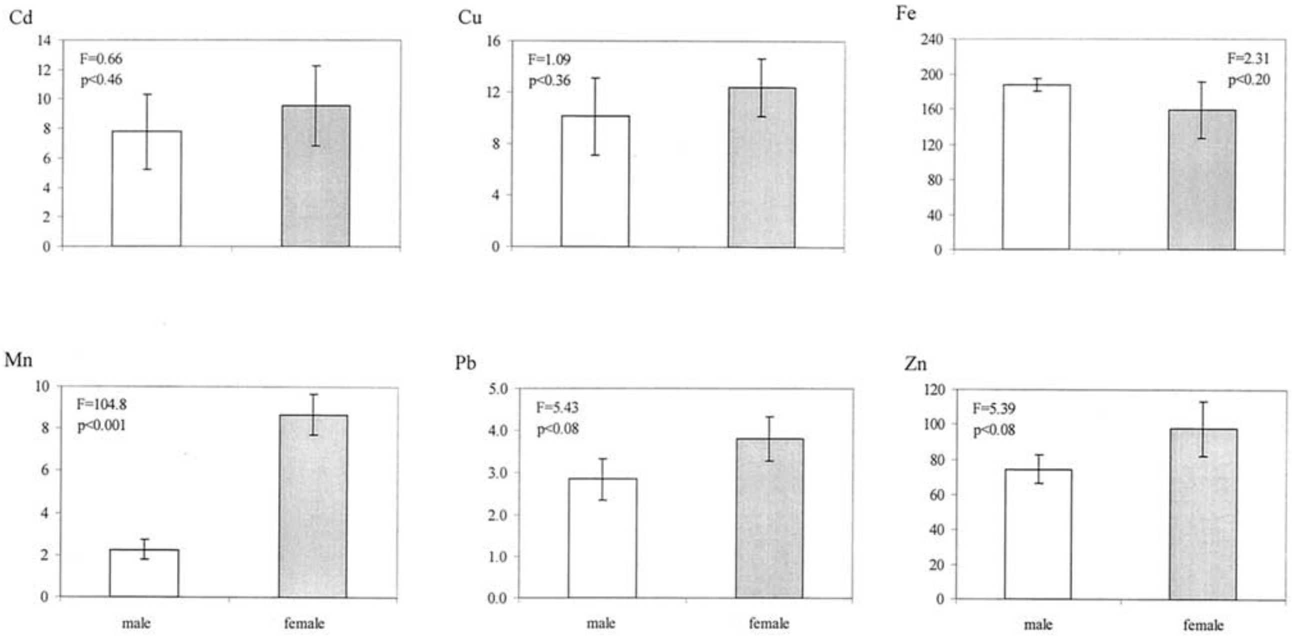

Fig. 8. Soft tissue concentrations of Cd, Cu, Fe, Mn, Pb, and Zn [$\mu g\ g^{-1}$ DW] in sex groups of *Perna perna* (mean ± SD; $n = 3$; inserts show results of *t*-test).

assessed on the basis of relations of metal concentration and metal content with the soft tissue and hydrological parameters. Relations of metal concentration and content to soft tissue dry weight revealed differences in behavior between the elements.

Cu, Mn, and Pb. Higher Cu, Mn, and Pb concentrations (and contents) in the brown mussel occurred between December and January (Figures 3 and 4), coinciding with an increase in tissue weight (Figure 5, Table 4). Winter metal accumulation may have derived from elevated concentrations of Cu, Mn, and Pb in the environment during the northeast (NE) monsoon. The period of NE monsoon is known as a rainy season during which winds are funnelled up the Gulf of Aden from the Arabian Sea into the Red Sea bringing torrential rainfalls and occasionally dust-storms. Although the precipitation occurs often in short bursts, it gives rise to seaward alluvial fans that characterise wadi discharges. Thus, the inflow of freshwater to the coastal zone is extended over the following few weeks because of the extensive and numerous wadi systems along the coastline (USOCD 1982). The wadies collect water through branched drainage canals from large inland areas and discharge it to the sea, often with great quantities of terrestrial sedimentary materials. By this route, coastal waters receive huge loads of suspended matter and consistently large loads of particle-associated elements of various kinds including metals, that can originate from natural sources (processes of chemical weathering and geochemical activity) and/or from local pollution sources (*e.g.*, domestic wastes, agriculture, etc.). In addition, the winter increase in tissue concentrations of Cu, Mn, and Pb may have been associated with faster metal uptake during intense food uptake as evidenced by a sharp rise in the dry weight and the rapid growth of the mussels in this period (Figure 5). After the main spawning season, which usually takes place from July to October, the organism starts to build up high-energy reserves in the form of carbohydrates (glycogen) and lipids by increased feeding (Bawazir 2000). The efficiency of absorption is then high due to elevated concentrations of suspended particles in the water column and relatively low water temperature, as observed also in the Multiple Regression Analysis (Table 5). It has been shown that in the tropical and subtropical regions, the efficiency with which mussels absorb filtered material and growth rate may be depressed by high temperature (Velez and Epifanio 1981; Lee 1986)

A subsequent decrease in Cu, Mn, and Pb soft tissue concentrations occurred in early spring (February through March), most probably as a response to the lower environmental concentrations of the elements during the seasonal transition period. The lack of vertical mixing of the seawater over this time period leads to diminishing amounts of suspended matter and in consequence to a reduction in metal availability (Murty and El-Sabh 1984). For Cu, and to a lesser extent Mn, a peak accumulated metal concentration was also recorded in October (Figure 2). A simple explanation favors the assumption that increased metal accumulation reflects higher levels of the metals in the environment, presumably caused by flushing away of the elements from beaches by severe wave action. This phenomenon moves much of the sand offshore, exposing areas of the calcareous platform and other rocky outcrops, and also scattered areas of fine gravel (Banaimoon 1986). The plots of Mn, Cu, and Pb concentration versus body weight (for the two size classes separately and combined) showed a positive trend, suggesting that *P. perna* has limited powers of excretion and tends to store metals with increasing weight (the weak correlation for Mn is due to considerable differences in concentrations and body weight between the two size classes; Table 4). In the case of Pb, which was the only metal with a positive significant correlation between both metal concentration and metal content with soft tissue dry weight, net accumulation with increase in body weight over time cannot be excluded either. It has been shown in many mollusc species that a significant correlation between metal content and body weight (with a slope greater than 1) might indicate a net metal accumulation strategy (Rainbow 1993). Thus, the concentration of Cu and Mn in the tissue of *P. perna* seems to be roughly proportional to the environmental availability providing evidence for the good biomonitoring capacity of the mussel for these elements, while the level of Pb can be related with body weight.

Fe and Cd. Compared to Cu, Mn, and Pb, the behaviors of Fe and Cd appeared to be more complex. Seasonal variations in Fe and Cd concentrations were in general accompanied by changes in soft tissue dry weight. Plotting metal concentration versus body weight showed negatively sloped relationships (although not statistically significant either in the two size classes separately or combined; Table 4), hence suggesting that biological processes are important factors in the possible regulation of metal loads in the mussels to approximately constant levels. Marked decreases in tissue levels of Fe and Cd occurred in winter (December through February) during the period of raised body weight, while an increase in metal concentration in spring (March), and for Cd additionally in November, coincided with tissue weight loss (Figures 2, 4, and 5). Tissue dilution of the accumulated metal concentration due to an increase in weight followed by a decrease in metal concentration by increasing tissue weight probably provides the explanation. The same effect has been already reported for other bivalve species of the family *Mytilidae* in boreal and tropical zones: *Mytilus edulis* (Amiard *et al.* 1986; Borchardt *et al.* 1988), *Mytilus trossulus* (Kavun 1991), and *Perna viridis* (Lakshmanan and Nambisan 1989). This overall pattern might be temporally modified by a simultaneous action of various environmental factors affecting metal availability as suggested also by the regression of metal contents against soft tissue dry weight (Table 4). The regressions of Cd and Fe contents with body weight were positively sloped for the two size classes combined, implying a connection with metabolism. However, the trends changed to negative when the size classes were analyzed separately indicating that some external factors could well be influencing metal level (Boyden 1974). This seems to be the case for Fe and Cd levels in the brown mussels from the Yemeni coastal area. For example, an exceptionally high tissue concentration of Fe (up to 570.5 $\mu g\ g^{-1}$ DW) appeared in October, similarly to Cu and Mn, most likely on account of elevated exposure caused by influx of Fe to the shallow coastal waters (Banaimoon 1986). Iron is very insoluble in the sea water and exists mainly in a form associated with lithogenic particles. Its particulate concentration often increases with increasing contribution of lithogenic material in suspension. Extremely low particulate organic matter (POM) in the intertidal zone of the Gulf of Aden was observed in October 1997 (1 mg dm^{-3}), indicating a high load of terrigenic nonorganic material in the water column in this season and presumably an elevated Fe level. For Cd, an unexpected increase in tissue concentration was recorded in September. This may have been connected to the end of the SW monsoon with strong upwelling resulting in the injection of nutrients into the euphotic zone and high productivity as confirmed also by Multiple Regression Analysis (Table 5). There is evidence that the Indian Ocean deep waters are rich in elements such as Cd, concentrations of which are depleted by biological processes in the surface layers and increase with depth. For instance, Nolting *et al.* (1989) observed during the Snellius II expedition that nutrient enrichment (particularly of phosphates) in the coastal waters of eastern Indian Ocean (off Indonesia) during upwelling was accompanied by a distinct increase in dissolved and particulate-associated Cd concentrations, thus exposing local faunal communities to elevated metal environmental concentrations.

Although the contributing effects of biological (internal) and environmental (external) factors on Fe and Cd accumulation in brown mussels still remains hard to define, the data presented here clearly demonstrated the influence of both parameters on metal levels in the mussel. Seasonal variation of these elements caused by changes in body weight can be overlapped and masked by the large environmental fluctuations brought about by short-term metal enrichment in seawater.

Zn. The tissue concentration of Zn displayed, similarly to Fe and Cd, slightly negative relationship with body weight (Figure 6, Table 4), indicating the possible existence of the metal equilibrium in the mussel. Bryan (1984) suggested that the metal concentration in the body remains constant or falls with increasing size (weight) when the organism excretes the metal at a rate proportional to the body burden and thus the body level is controlled by metabolic processes. According to Boyden (1974), a function of the body weight such as the extent of binding of specific components within the tissue plays some role in determining the total metal burden in bivalves when the element content is directly related to body weight. Indeed, a significant linear correlation between metal content (body burden) and soft tissue body weight was apparent for Zn, but not for Fe and Cd (Table 4), providing additional support to the contribution of internal mechanisms in Zn regulation. Metabolic regulation of the accumulated Zn concentration to an approximately constant level, making this accumulated concentration to some degree independent of its environmental levels, has been observed as a common feature in various animals. Physiological control has been suggested as a key mechanism responsible for maintaining constant Zn tissue concentrations in different estuarine invertebrates including polychaetes, *e.g.*, *Nereis diversicolor* (Amiard *et al.* 1987), bivalves such as *Scrobicularia plana* (Amiard *et al.* 1987), *Perna viridis* (Phillips 1985; Phillips and Rainbow 1988; Chu *et al.* 1990), and *Mytilus edulis* (Lobel *et al.* 1989), and gastropods, *e.g.*, *Littorina littorea* (Bryan 1984). The most common method of Zn regulation in mussels is via metal excretion with mucus, faeces, or as granules from the kidney, thereby maintaining a relatively constant body content, presumably equivalent to physiological need (George and Pirie 1980; Rainbow 1996). Binding to specific low-molecular-weight proteins of the metallothionein type, which are not involved in normal cell metabolism but may transfer Zn to apoenzymes required in many metabolic pathways, provides an alternative (George and Langston 1994). The brown mussel can be considered a potential regulator of Zn level in its tissue. Biological control of the accumulated Zn concentration seems to be reflected to some extent by its seasonal pattern. Zn concentrations gradually increased over nearly the whole period studied with only minor fluctuations, in spite of considerable temporal changes in metal exposure and variations in environmental conditions. The maximum level of Zn concentration occurred in spring (March), presumably as a concentrating effect of decreasing (in size class B) body weight, the phenomenon already being observed for Fe and Cd and confirmed by the negative slopes of the relationship of metal concentration with soft tissue dry weight (Table 4). Tissue Zn concentration in *P. perna* appeared, therefore, to be mainly driven by biological processes with sporadic external influence.

The effects of spawning on Zn concentration (and content) of soft tissues described for some bivalve species (Mauri and Orlando 1983; Karaseva 1993) were not readily apparent in this study. Bawazir (2000) observed continuous reproductive activity of *P. perna* from the Gulf of Aden (off Yemen) throughout the whole year with peak spawning in July through September. Body weights and Zn concentrations of mature individuals (in both size classes) did not display any consistent change during these months. Lobel and Wright (1982) also have related the seasonal dynamics of Zn in *Mytilus edulis* to fluctuations in the weight of somatic tissue and/or environmental exposure rather than to gonad development and release of gametes. Similarly, Karaseva (1993) reported that Zn accumulated in gonads was mainly associated not with reproductive cells (sperms and ovaries) but with somatic gonadal cells.

The Effect of Sex

Although fluctuations of metal accumulation in mussels with sex have been widely studied in a variety of species employing both field and experimental approaches, the overall concept of this relation is still debatable. Watling and Watling (1976), for example, reported markedly higher concentrations of Mn, Cu, and Zn in females of *Choromytilus meridionalis* from the South African coast, while no significant difference were observed for Fe, Pb, and Cd. Cu proved to be more concentrated in females of *Mytilus californianus* from Southern California Bight (Gordon 1978) and Zn in females of *Perna viridis* from the Bay of Bengal (Chidambaram 1992). On the contrary, Kavun (1991) noticed no intersex differences in metal concentration in three mytilid species in the northwestern Pacific.

In *P. perna* from the coastal zone off Yemen, all metals, except Fe, exhibited greater tissue concentrations in females than in males, a statistically significant difference being proved for Mn (Figure 5). This may have been attributable to the changes in soft tissue dry weight related to the development cycle of gonads which reach the ripening stage as early as in July in both sexes (Bawazir 2000). Orren *et al.* (1980), analyzing the black mussel *Choromytilus meridionalis* separated into two sex groups, observed that Mn, Fe, Cu, and Zn were more concentrated in females only before spawning. After the main breeding period no differences were found, suggesting reproduction-related metal accumulation in the bivalves. Gonad maturation in spring implies certainly an increase in the amount of gonadal tissue and concurrent storage of lipids and protein in sexual products as the main energy and structural materials, respectively. Since the contribution of the amount of reproductive tissues to the total body weight in females exceeds that in males (Lobel and Wright 1982), formation of reserves during the prespawning period becomes more pronounced in females (Pieters *et al.* 1980), favoring faster metal accumulation in this sex. Furthermore, Karaseva (1993) in laboratory experiments on related *Mytilus trossulus* and *Crenomytilus graganus* from Peter the Great Bay (Sea of Japan) recorded considerably higher Zn concentrations in gonads of females compared to those of males. Although the results of this study do not prove involvement of gametogenetic processes in metal accumulation, the role of the gonads in determining metal tissue concentrations cannot be neglected and needs further study. The effect of mussel size on metal concentration is of rather minor importance in this study because the shell length of animals was restricted to a narrow range of 50–59 mm.

Conclusions

In summary, the present study has shown that concentrations of Cd, Cu, Fe, Mn, Pb, and Zn in soft tissues of the brown mussel *P. perna* from the coastal waters off Yemen fluctuate temporally with variable amplitude. Seasonal patterns differ among metals depending on the specific behavior. Mn and Cu soft tissue accumulated concentrations appear to be sensitive to environmental conditions including levels of metal exposure, and thus seem to reflect ambient metal availability in the ecosystem quite efficiently. The behaviors of Fe, Cd, and partially Pb are influenced by both biological processes and environmental exposure, and the extent of a certain effect in determining metal level varies with the actual environmental conditions in relation to seasonal cycle. The concentration of Zn is mainly controlled by internal mechanisms of an organism with a limited contribution of environmental metal level. The effect of seasonal evolution of body weight on soft tissue metal concentration has been identified for Fe, Cd, and partly Zn. Intersex differences were noticed for all elements. It has been suggested that this might result from the different rate of gonadal development in the prespawning period and consequently a large disproportion in the amount of gonadal tissue formed between the two sexes.

Finally, it is suggested that *P. perna* may be used successfully to monitor Mn and Cu availability in coastal waters.

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