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VARIATIONS IN TRACE METAL CONTENT OF THE MUSSEL *Mytilus galloprovincialis* Lamark WITH SEASON AND SIZE

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ABSTRACT: *Shells and soft tissues of two different size groups of mussels* (M. galloprovincialis) *collected seasonally (July 1989, October 1989, January 1990 and April 1990) from Rumeli Fort were analyzed for Zn, Cu and Hg, Hg was analyzed only in soft tissues. Concentrations of these metals were correlated with sizes and seasons.*

Significant seasonal changes in the concentrations of Cu were not observed in both sizes of shell samples. Maximum Zn concentrations in shells of small sized individuals were observed in summer, although big ones didn't show any significant seasonal changes.

In soft parts of both size groups highest concentrations of Zn and Cu were found in autumn. Maximum Hg concentration was observed in autumn in soft tissues of big sized mussels, in small sized individuals however the highest Hg concentration was measured in summer.

Key words: Pollution, Trace metals, Mussel, Mytilus galloprovincialis

MİDYENİN *Mytilus galloprovincialis* Lamark İZ METAL İÇERİĞİNİN BOY VE MEVSİMLERE GÖRE DEĞİŞİMİ

ÖZET: *Rumeli Hisarı'ndan mevsimsel olarak (Temmuz 1989, Ekim 1989, Ocak 1990 ve Nisan 1990) iki farklı boy gruplarında toplanan midyelerin et ve kabuk örneklerinde Zn, Cu ve Hg analizleri yapıldı. Hg analizi yalnızca et örneklerinde yapılmıştır. Örneklerdeki metal miktarlarının boy ve mevsimlere göre ilişkileri saptandı.*

Cu miktarları her iki boy gruplarındaki kabuk örneklerinde önemli mevsimsel değişim göstermemiştir. Büyük midyeler Zn miktarında önemli bir mevsimsel değişim göstermemesine rağmen, küçük midyelerde en yüksek Zn miktarı yazın gözlenmiştir.

Küçük ve büyük boy midyelerin etlerindeki en yüksek Cu ve Zn miktarları sonbaharda bulunmuştur. En fazla Hg miktarı büyüklerde sonbaharda gözlenirken, küçüklerde yazın bulunmuştur.

Anahtar Kelimeler: Kirlilik, İz metaller, Midye, Mytilus galloprovincialis.

INTRODUCTION

Oceanic waters and especially inshore waters, are increasingly loaded with pollutants. Heavy metals are one group of these pollutants which has significant influence on marine life.

Marine pollution monitoring programmes commonly employ bivalve molluscs as indicator organisms to assess the degree of heavy metal pollution in their surrounding environment because of their sedentaryness, wide range distribution and also feeding mechanisms (suspended feeders) (1). Environmental metal level is not the only factor affecting the metal content of mussels as both the size of mussels (2-3) and the season markedly affect this parameter (4-5-6).

Present paper examines the relationship between trace metal content, shell length and body dry weight for two different size groups of *Mytilus galloprovincialis* collected from Rumeli Fort in Istanbul in different seasons.

MATERIAL AND METHODS

Collected mussel samples were classified according to their size; 1-4 cm were considered as small size and 6-8 cm as big size. The composite samples contained the soft parts of three mussels from each size group. The shells of mussels were opened by a clean plastic knife inserted into the adductor muscles according to the method described by Bernhard (7).

Certified samples obtained from Community Bureacu of References (BCR) were used for the verification and the calibration of the methods.

Digestion of Soft Parts Samples

Approximately 1g of sample was placed into a teflon digestion vessel. 3ml concentrated nitric acid were added for the digestion of organic matrix and then the vessel was closed. Teflon crucibles were placed in a stainless steel block. The steel block was heated to 130^{o}C on a thermostatic controlled hot plate for 19 hours. Then the steel block was removed from the oven and let cool to room temperature. After this procedure, the digested samples were diluted to 25 ml with deionized distilled water. The heavy metal concentrations in these solutions were determined by Atomic Absorption Spectrophotometer (Varian-Techtron Model AA6 and Model 1250).

Digestion of Shell Samples

The shell samples were first dried at 50^{o}C for 24 hours, then weighed and graved in a mortar to homogenize. After homogenization, samples of 0.4-0.7 g were weighed, transferred to the teflon vessels and then 3ml concentrated nitric acid was added. The solution was heated for 8 hours until about its dryness and then 3ml of nitric acid and 1ml of concentrated perchcloric acid were added in order to digest the organic materials. After readding 3ml of concentrated nitric acid to eliminate the chlorite, the solution was allowed to evaporate. Finally, 2 ml of nitric acid was added and the sample was diluted to 10ml with deionize distilled water (Modified from 8).

RESULTS AND DISCUSSION

The shell samples were analysed for their Cu and Zn contents while concentration of Hg was also measured in addition to Zn and Cu in flesh samples.

The seasonal fluctuations in temperature and salinity in the studied region and the summary of analytical results are given in Table 1.

Table 1. Seasonal Fluctuations in Temperature and Salinity and Average Concentrations (Mean+SD in DW) of Cu, Zn and Hg in Shell and Soft Tissues of *Mytilus galloprovincialis*.

	Temparature	Salinity	Shell length	copper (μg/g)		zinc (μg/g)		mercury (ng/g)
seasons	^{0}C	psu	(cm)	soft part	shell	soft part	shell	soft part
summer	23.5	17.7	S=3.4±0.2	14.7±3.8	0.2±0.01	255.5±43.2	3.7±0.8	342.7±57.3
			B=3.4±0.2	7.2±1.0	0.1±0.08	207.6±65.1	1.4±0.1	115.2±30.9
autumn	16.5	16.0	S=3.5±0.2	14.9±1.3	0.2±0.1	432.5±70.1	2.0±0.4	145.3±17.8
			B=7.1±0.5	13.4±1.8	0.2±0.03	966.6±228.8	1.6±0.3	194.2±53.0
winter	5.0	18.3	S=3.4±0.1	8.2±1.1	0.3±0.02	238.3±105.3	2.7±0.2	104.0±12.8
			B=6.7±0.4	6.3±1.4	0.1±0.06	311.4±181.4	1.6±0.5	109.7±16.3
spring	9.2	17.3	S=3.5±0.3	8.4±1.0	0.4±0.2	271.0±170.8	1.9±0.6	106.4±24.1
			B=6.6±0.4	8.4±1.4	0.1±0.01	371.1±184.	1.4±0.2	96.2±19.1

DW = Dry Weight S = Small size B = Big size

In order to determine whether metal concentrations differ among seasons for the two size groups, a single classification analysis of variance is performed and results are summarized in Table 2.

Seasonal Variations

Zinc

As can be seen from Tables 1 and 2, whilst the concentrations of Zn were higher in small-sized shell samples in summer, no significant seasonal changes could be detected in big-sized ones. Zn concentration in soft tissues was higher in autumn in both size groups.

Copper

There is no significant seasonal changes for Cu concentrations in both size groups of shell samples (Table 2). Cu accumulation in soft tissues of the small-sized organisms were found to be higher in the summer and autumn months while high levels of this metal were observed in soft tissues of big-sized mussels in autumn. (Table 1 and 2).

Table 2. Single Classification of ANOVA Table for the Seasonal Concentrations of Cu, Zn ang Hg.

Metal	Size	Source of Variation	Soft Tissues Fs	Shell Fs
Cu	B	among seasons	$F_{[3,21]}$ = 26.45**	$F_{[3,8]}$ = 0.64
	S	among seasons	$F_{[3,21]}$ = 16.08**	$F_{[3,8]}$ = 0.94
Zn	B	among seasons	$F_{[3,20]}$ = 17.83**	$F_{[3,8]}$ = 0.20
	S	among seasons	$F_{[3,21]}$ = 3.54*	$F_{[3,8]}$ = 4.51*
Hg	B	among seasons	$F_{[3,23]}$ = 10.12**	-
	S	among seasons	$F_{[3,23]}$ = 68.38**	-

S = Small Size B = Big Size * = P<0.05 ** = P< 0.01

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In this study generally high concentrations of Cu and Zn in the soft parts of both size groups were found in autumn. This increase was probable due to the development of the gonads during this season. Segar et. al., (9) found that the concentrations of many metals in the gonads of molluscs were higher than in some other organs. Uysal (10) pointed out that the spawning of *M. galloprovincialis* occured from september to the end of may in Izmir Bay. This shows us in autumn mussels are in spawning period. Highest concentrations of most metals normally occur in kidneys if accumulation has taken place over a long period of time, while shorter term accumulation usually results in increased concentration in the gills (11). George and Pirie (cf. 4) have also suggested that, soluble Zn might be taken up by the mantle which was quickly transported to kidneys for storage an excretion. In this case, gonads may be acting as a transit point for Zn but not as an organ of accumulation.

Mercury

Maximum Hg concentration in big-sized mussels was obtained in autumn while in small-sized groups, the high value was observed in summer. Cunningham and Tripp (12) also simulated a temperature sensitive Hg enrichment during the summer months and Hg loss during the winter in the oyster *Crosostrea virginica*.

A certain amount of survey data also exists in which authors have reported the concentrations of Hg in both, soft parts and shell of bivalves taken from several sites (13-14). They showed that, in no case do profiles for metal levels in the shells match those for the same metal in the soft parts. However, this does not appear to be due to the regulation of metal levels in the shells. The most probable explanation is that, the soft parts and the shell of molluscs respond differently to various portions of the total trace metal load. Thus, for example, the uptake of trace elements by shells may occur by adsorption from solution, whereas those taken by soft parts are probably dominated in most cases by metals ingested with food. However, this explanation relies on the assumption that trace elements in the shell are derived mainly by surface adsorption from solution. In some cases at least this may not be the case, metals may actually be integrated into the matrix during the synthesis of the shell by the mantle. But these measurements are affected by the experimental errors. Bryan and Uysal (14) found that, washing the animal with jet of top water, removed adhering sediment from the shell, a more firmly attached coating containing metals remained. This could be removed by scrubbing the shell with a brush. Comparisons between washed and scrubbed valves from 4 cm animals showed that scrubbing removed and additional 20% of Mg, 50% of Cu, 60% of Fe and 70% of Zn.

Effect of Size on the Uptake of Metals

Zinc

The results of the multiple regression analysis showed (Table 3), significant negative correlations between Zn concentrations in the shell samples and length and weight in summer and in winter ($P<0.01$). However, in autumn and in spring no significant correlation was found between length, weight and Zn concentrations.

Table 3. Correlation of Zn Concentration with Length (cm) and with Dry Weight (g) in Shell and Soft Tissues of *Mytilus galloprovincialis*

Samples	Seasons	No of samples	Correlation length	coefficient weight
shell samples	summer	6	-0.90**	-0.88**
	autumn	6	-0.52	-0.55
	winter	6	-0.85**	-0.88**
	spring	6	-0.47	-0.47
	whole year	24	-0.65**	-0.60**
soft tissue samples	summer	12	-0.33	-0.34
	autumn	12	+0.85**	+0.78**
	winter	13	+0.114	+0.002
	spring	12	+0.27	+0.06
	whole year	49	+0.26	-0.17

** : Highly significant (99%)

During a period of one year, without considering the seasons, there was a negative significant correlation between Zn against length ($r = -0.65$; $P<0.01$) and weight ($r= -0.60$; $P<0.01$).

Table 3 shows also the correlation of zinc concentrations in the soft tissues with body dry weight and shell length of *Mytilus galloprovincialis*. According to the results of the multiple regression analysis, a positive significant correlation was found only in autumn between length ($r = 0.85$; $P<0.01$), weight ($r = 0.78$; $P< 0.01$) and Zn concentrations. During a period of one year, regardless of the season no significant relationship was found between weight, length and Zn concentration. Boyden (3) pointed out that Zn concentration was independent of body size in oysters.

Copper

When the effects of length and weight on the uptake of Cu in shell samples are considered, significant seasonal changes occurred only in winter (Table 4).

Table 4. Correlation of Cu Concentration with Length (cm) and with Dry Weight (g) in Shell and Soft Tissues of *Mytilus galloprovincialis*.

Samples	Seasons	No of samples	Correlation coefficient length	Correlation coefficient weight
shell samples	summer	6	-0.30	-0.21
	autumn	6	-0.49	-0.44
	winter	6	-0.88**	-0.84**
	spring	6	-0.60	-0.61
	whole year	24	-0.47*	-0.43*
soft tissue samples	summer	12	-0.80**	-0.76**
	autumn	12	-0.42	-0.28
	winter	13	-0.64*	-0.66*
	spring	12	+0.05	+0.18
	whole year	49	- 0.34*	-0.52**

* : Significant (95%)
** : Highly significant (99%)

In the soft tissues there was no significant correlation between length, weight and Cu concentrations in autumn and spring. In summer and in winter, negative significant correlation existed between length, dry weight and Cu concentrations. Without considering the seasons, negative significant correlations were found between length ($r= - 0.34$; $P<0.05$), weight (r= 0.52; P<0.01) and Cu concentrations. In general Cu showed negative significant correlations against length and weight in the present study. Boyden (3) also showed that small individuals equilibrate relatively rapidly (within 5 months) when exposed to enhanced environmental Cu concentrations, whilst large individuals do not adjust so rapidly.

As can be seen from Tables 3 and 4, the concentrations of Cu and Zn in the shell samples decrease with increasing size. The small size individuals have large surface to volume ratio. Trace metals in the shell are derived mainly by surface adsorption from the environment (15). This can be an explanation for the higher levels in small sized individuals.

Mercury

The results of the multiple regression analysis for Hg with length and also with body dry weight are shown in Table 5.

Table 5. Correlation of Hg Concentrations with Length (cm) and with Dry Weight (g) in Soft Tissues of *Mytilus galloprovincialis*

		correlation coefficient	
seasons	No of samples	length	weight
summer	12	-0.89**	-0.85**
autumn	12	+0.56*	+0.53*
winter	13	+0.11	-0.007
spring	12	-0.24	-0.24
Whole year	49	-0.22	-0.41**

* : Significant (95%)
** : Highly significant (99%)

There was no significant correlation between Hg-length and Hg-weight in winter and in spring. In summer negative significant correlations were found between length (r = - 0.89; P<0.01), weight (r= 0.85; P<0.01) and Hg concentrations. Positive significant relationships were found between dry weight, length and Hg concentrations (P<0.05) in autumn. A singificant correlation existed only between Hg concentration and body dry weight (r = - 0.41; P<0.01) if the seasons are not taken into consideration.

Dewolf (cf. 15) found that, mercury concentrations in the mussels, *M. edulis*, collected from two sites in Europe did not differ considerably with age. In general, authors studying the kinetics of Hg in bivalves maintained under laboratory conditions have found that, small individuals respond more rapidly to changes in ambient levels of mercury. The accumulation from solution was more rapid in small individuals than in larger ones in the oyster, *Crassostrea virginica* and the mussel, *M. galloprovincialis* (12). Therefore, smaller individuals appear essentially to time-integrate ambient Hg levels over a shorter period compared with large bivalves of the same species. Also smaller individuals have a greater surface area to volume ratios than larger mussels. Because of the possible surface surption effects, the trend of enhanced Hg uptake by smaller individuals must be examined in the soft tissues of the mussels before carrying out any metabolic observation, as has been suggested on the uptake of other heavy metals by *M. edulis* (2).

Haugh et. al. reported that (cf. 16) the uptake of metals by mussels in proportional to the metal concentrations in the environment. It is generally belived that, bivalves are excellent indicators of heavy metal pollution since the internal tissue concentrations often reflect increases in the environmental concentrations.

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