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ORIGINAL ARTICLE

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Seasonal variations of arsenic in mussels *Mytilus galloprovincialis*

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Abstract Total arsenic concentration in the edible part of mussels *Mytilus galloprovincialis* was evaluated seasonally in the coastal area of Rijeka Bay (North Adriatic Sea, Croatia). Sampling stations were located close to the City of Bakar with no industrial facilities (site 1), in the vicinity of the oil refinery and oil thermoelectric power plant (Urinj, site 2), and 4 miles away from the Plomin coal thermoelectric power plant (Brseč village, site 3). Additionally, the concentration of arsenic in the tail muscle of the lobster *Nephrops norvegicus*, collected in Rijeka Bay, was studied. During winter at sites 2 and 3, the total arsenic in the edible part of the mussels was 16.4 mg As/kg FW (FW=fresh weight) and 4.38 mg As/kg FW, respectively, and increased during springtime at site 2 (6.5 mg As/kg FW) compared to the rest of the year, when individual total arsenic concentration at all sites ranged from 1.7 to 3.7 mg As/kg FW. In the winter (sites 2 and 3) and springtime (site 2) there was no correlation between the length of the mussel shell and the arsenic concentration in the edible part of the mussels. In the other seasons, at sites 1, 2 and 3, there was a correlation between arsenic in the edible part of mussels and shell length in most cases (correlation coefficients r varied from 0.64 to 0.85; $P < 0.05$ to $P < 0.01$). Correlation between shell length (in the narrow range of shell lengths from 3.4 to 5.0 cm) and arsenic in the edible part of the mussels shows linearity with a high regression coefficient ($r = 0.914$; $P < 0.001$). The increase of arsenic in the mussels during winter and spring was suggested at least partially as a result of a low nutritional status, i.e. reduced weight of the mussels' edible part during winter. In addition, a linear relationship was found between body length and arsenic concentration in the tail muscle (mean 17.11±4.48 mg As/kg FW) of the Norway lobster.

Keywords Arsenic · *Mytilus galloprovincialis* · Rijeka Bay

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Introduction

The aquatic environment is important in the global cycling of arsenic. The background level of arsenic in marine environments is slightly higher (2–3 μg/l) than in freshwater and terrestrial environments (Moore and Ramamoorthy 1985; Phillips 1990). Atmospheric deposits, output from rivers, and up-welling from marine sediments lead to enrichment of arsenic in marine organisms. The arsenic content in the body results from the balance between the processes of metal accumulation and depuration. The dominant form of arsenic in marine and brackish waters is arsenate. Tissues of marine invertebrates accumulate arsenic in the range of 1–100 mg/kg DW (DW= dry weight), mostly in the form of organoarsenic (arsenobetaine) compounds. Up to 22% of the total arsenic is occasionally present as inorganic As (Neff 1997).

Arsenobetaine and arsenocholine are more efficiently accumulated from seawater by the mussel *Mytilus edulis* than other chemical forms such as arsenite and arsenate and other organometallic complexes (Gaisler et al. 1995). Arsenobetaine has been found to be the major organoarsenic compound in the muscle of freshwater fish (Shiomi et al. 1995). Excretion of dimethylarsinic acid in urine was significantly higher in volunteers following consumption of mussels containing high arsenic concentrations than would be expected on the basis of the present methylated derivatives in shellfish (Buchet et al. 1994).

Human consumers bioaccumulate mostly arsenobetaine, which is neither toxic nor carcinogenic to mammals, and therefore represents a low risk to the consumers

of fishery products (Neff 1997). Not more than 2–10% of the total arsenic is potentially harmful as a toxic form (Fowler 1983; Freiberg 1988). The mean concentration of the total arsenic in molluscs was found to be 11.1±3.4 μg/g dry weight (the fresh weight value is about 5-fold less; O'Connor 1998). Molluscs are often used as organisms in which concentrations of metals in tissues can reflect their content in the aquatic environment. Most of the arsenic taken up from seawater by invertebrates is retained in the gills and midgut gland. For example, the content of arsenic ranges from 36 to 100 mg/kg FW (FW=fresh weight) in the gills of *Carcinus maenas* (Andersen and Depledge 1994).

The objective of this study was to determine seasonal variations in arsenic levels in the mussels sampled in two North Adriatic coastal regions close to a coal power plant and refinery, and in a region where no industrial facilities are present.

Additionally, the muscle arsenic content of the Norwegian lobster *Nephrops norvegicus*, which is a representative organism living on the clay-loamy bottoms of Rijeka Bay in the North Adriatic, was studied.

Materials and Methods

Materials

The mussels *Mytilus galloprovincialis* (L.) were collected from three sampling stations along the North Adriatic coast of Rijeka Bay and the east coast of the Istrian peninsula during 1998 and 1999. Two sampling stations were close to the city of Rijeka: one in the coastal region where there are no industrial facilities (Site 1; Bakar), and the other in the vicinity of an oil refinery and thermoelectric power station (Site 2; Urinj). The third station was located on the east coast of the Istrian peninsula (Site 3; Brseč), 4 miles away from a coal-fired power plant. The shell length of the sampled mussels ranged from 2.5 to 4.3 cm. The mussels used for experimental purposes were out of the reproductive cycle.

Norway lobster, *N. norvegicus* , were caught during the summer using traps in Rijeka Bay. The animals were transferred to laboratory aquaria and kept in running seawater for a few days before the experimental procedure.

Preparation of tissue and arsenic measurement

Fresh weight of the tissues (1.5 g) was added to 5 ml ultra pure HNO_3 (concentrated high grade chemicals) in teflon reaction vessels. Microwave oven combustion was performed for 22 min in Teflon chambers MLS 1200 MEGA "exhaust module" EM-45/A (Milestone, Italy) (Legarde et al. 1999). Digested tissue was diluted with double distilled water up to 100 ml.

A Perkin-Elmer PE 4110 ZL atomic absorption spectrometer (AAS) was supplied with transverse-heated graphite tubes and cups (PE-B 300-0638). Arsenic standards 1 mg/ml N930-01800 PI (Perkin Elmer) were used. Arsenic was determined at a wavelength of 193.7 nm, slit=0.7 nm with As-EDL source of irradiation with gas argon. The accuracy of the method was checked by measuring the arsenic in a mussel tissue sample certified by IAEA, Monaco. The mean values of As concentration were in the range of the certified values. Calibration curves from standard mixtures of arsenic were prepared in nitric acid solution. The accuracy of the method was determined by preparing digestion mixture blanks and by spiking the samples with known concentrations of arsenic.

Statistical analyses

Calculated values are expressed as means±SD of two sample groups to determine whether differences between them are significant (Quattro Pro Computer test). Unpaired two-tailed Student *t*-tests were performed. *P* values of <0.05 were considered significant. Significant differences between treatments were assessed using ANOVA in combination with the Tukey test. Normality and homogeneity of variances assumption were checked (Zar 1999).

Results and discussion

In the mussels *M. galloprovincialis* collected seasonally from the off-shore subtidal sites of the North Adriatic Sea (Fig. 1), the total arsenic concentration in the edible part was determined (Table 1). At site 1, close to the city of Bakar, no large seasonal fluctuations were found in the total arsenic concentration (2.0–3.2 mg/kg FW). The results agree with those obtained for total arsenic monitored for the mussel *M. edulis* over more than 10 years by the National Status and Trends Programme on the US coast. Considering that dry weight approximately equals fresh weight/5, the values in that study of 8.2 to 10.14 mg As/kg DW are consistent with values reported here, and showed no upward trends during the study period (O'Connor 1998). A significant linear relationship was found between shell length and total arsenic concentration in the edible part in all seasons ($P<0.05$ or $P<0.01$; Fig. 2). Increased total arsenic levels of 16.4 and 4.4 mg As/kg FW were found in winter at sites 2 and 3 (close to the industrial facilities), respectively, site 2 keeping a high level during spring (6.5 mg As/kg FW). At sites 2 and 3 during the rest of the year, total arsenic ranged from 1.7 to 3.7 mg As/kg FW, values which are within the range of those at site 1 (Table 1). Moreover, at sites 2 and 3, no correlation was found between shell length and total arsenic in malnourished winter and spring populations of mussels ($P>0.05$; Fig. 3). During the rest of the year, a correlation was found between total arsenic in edible parts with overall shell length (site 3 and summer, site 2) at a level of significance similar to those at site 1 ($P<0.01$ and $P<0.05$; Fig. 3).

Tissues of marine invertebrates, including bivalve molluscs from non-polluted areas, contain concentrations of total arsenic ranging from 0.2 to 20 mg/kg FW (UNEP 1978; Langston 1980; Benson and Summons 1981; Neff 1997). These values are in the range of those described here, including values during winter and spring close to the industrial facilities, and therefore would not be exclusively related to the anthropogenic sources.

In our experiments, a narrow range of shell lengths (3.4 to 5.0 cm) linearly correlates with the fresh weight of the edible part of mussels out of their reproductive cycle ($r=0.914$; $P<0.001$; Fig. 4). These results are in accordance with the approximation that the growth rate of young stages of *M. edulis* is high up to 6 cm shell length, and that resource allocation depends on trophic availability, gonad activity and physiological status (Hawkins and Bayne 1992). The relationship between shell length (i.e. growth of mussels) and arsenic content in the edible

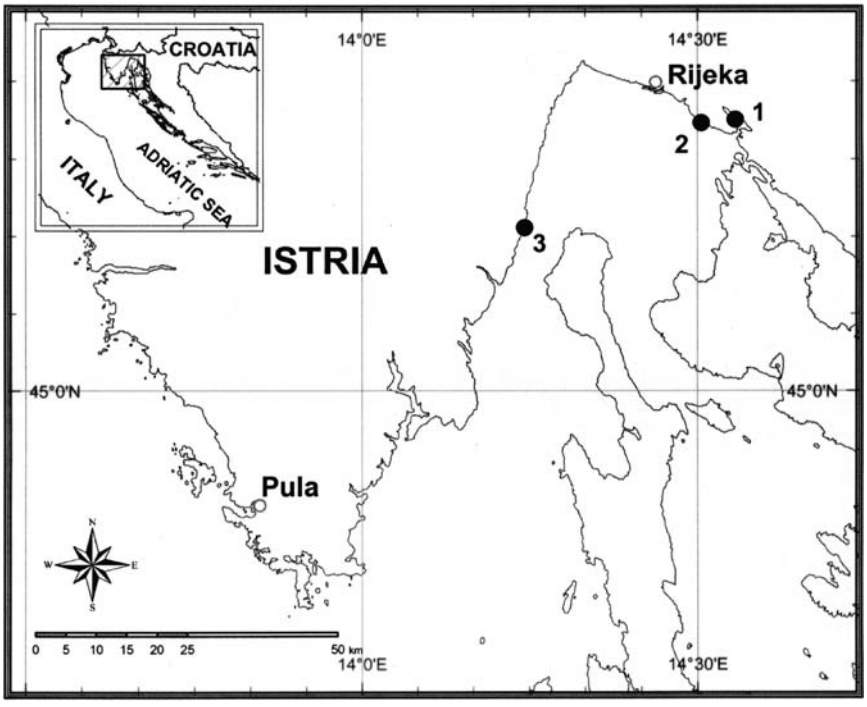

Fig. 1 Sampling sites of the mussels *Mytilus galloprovincialis* in the coastal region of the North Adriatic Sea. Site 1 (city of Bakar), site 2 (Urinj; vicinity of thermoelectric power station and oil refinery), site 3 (Brseč; 4 miles away from Plomin coal-fired thermoelectric power plant)

Table 1 Concentration of arsenic (mg/kg fresh weight) in edible part of the mussel *Mytilus galloprovincialis* taken from offshore along the Northern Adriatic coast in Rijeka Bay (Croatia). Means±SD and number of samples (in *brackets*); minimum–maximum shell lengths (cm) also in brackets

	Site 1 (Bakar)	Site 2 (Urinj)	Site 3 (Brseč)
March 1999	2.02±0.15 (8) (3.9–4.2)	16.37±7.46 (10) (3.5–4.0)	4.38±0.35 (10) (3.6–4.3)
April 1999	2.51±0.24 (8) (3.5–3.7)	6.46±0.48 (9) (2.9–3.2)	2.94±0.17 (9) (3.5–4.1)
July/August 1998	3.23±0.55 (9) (2.8–3.4)	3.02±0.48 (10) (3.3–3.6)	3.74±0.20 (8) (2.5–4.5)
September/ October 1998	2.03±0.08 (7) (3.4–3.6)	1.67±0.12 (10) (2.7–3.7)	2.59±0.42 (10) (2.6–2.7)

part of the mussels suggests that much of the total arsenic is bioaccumulated by water filtration and food assimilation.

Increased concentration of metals in soft tissues in winter and spring relative to other seasons has been found for *M. edulis* (Phillips 1976; Amiard et al. 1986), *M. galloprovincialis* (Majori et al. 1978) and *Macoma balthica* (Bordin et al. 1992). There could be several explanations for increased arsenic concentration in winter compared to the other seasons. First, reduced dry weight of the edible part of *M. galloprovincialis* with respect to shell weight shows the decrease in soft tissue mass during the winter, when increased metal concentration was recorded (Soto et al. 1995). Due to decreased food assimilation in winter, the nutritional status of mussels drops, the edible body mass decreases, and the metal content remains the same, but concentration expressed in relation to edible body mass therefore increases.

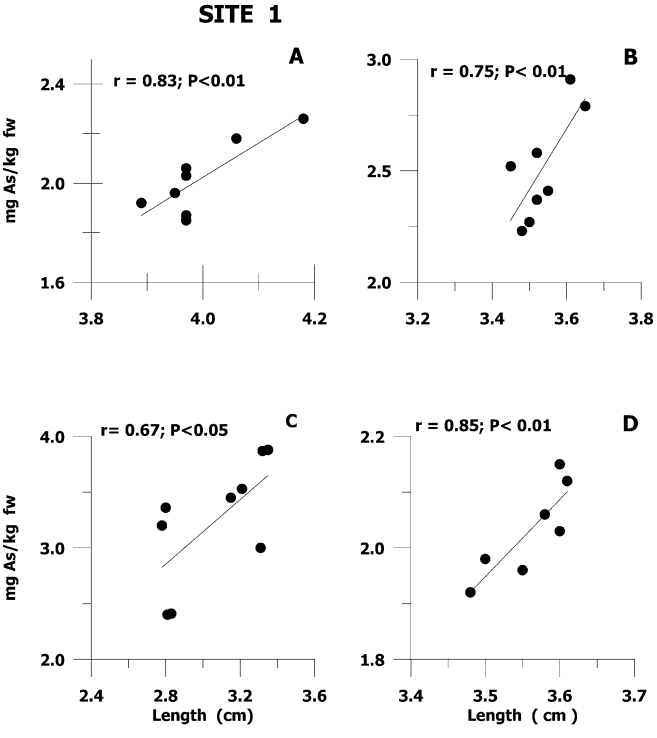

Fig. 2A–D Correlation of arsenic in edible part of the mussels (mg As/kg FW) and shell length of mussels *M. galloprovincialis* collected seasonally at site 1 (city of Bakar). **A** winter; **B** spring; **C** summer; **D** autumn

In the region of the oil refinery, enhanced bioaccumulation of arsenic may result from an increase in nutrients in late winter and early spring due to increased freshwater inputs (Ecology study in the Rijeka Bay 1977).

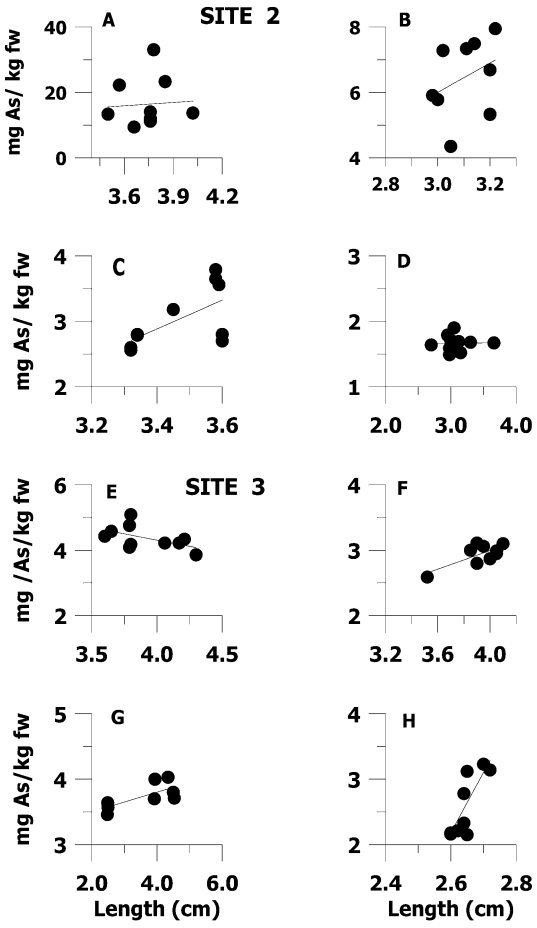

Fig. 3A–H Correlation of arsenic in edible part with shell length of mussels *M. galloprovincialis* collected at sites 2 (Urinj; **A–D**) and 3 (Brseč; **E–H**). **A** winter (r=0.21; P>0.05); **B** spring (r=0.35; P>0.05); **C** summer (r=0.64; P<0.05); **D** autumn (r=0.02; P >0.05); **E** winter (r=0.53; P>0.05); **F** spring (r=0.77; P<0.01); **G** summer (r=0.72; P<0.01); **H** autumn (r=0.79; P<0.01)

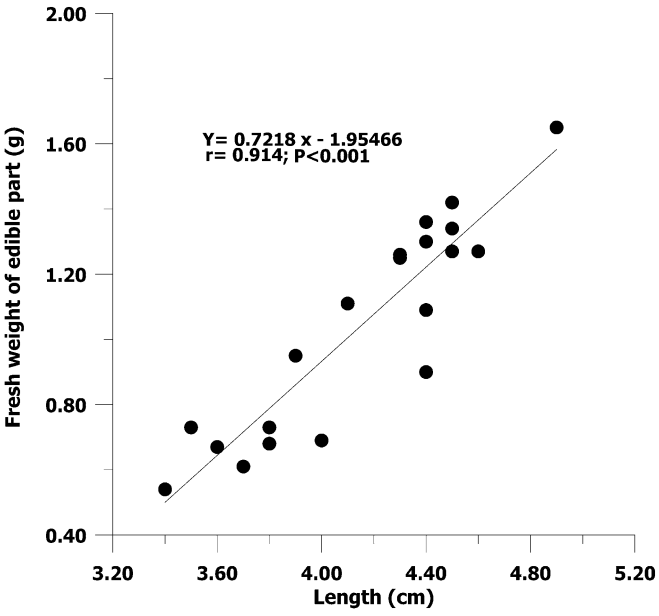

Fig. 4 Correlation between fresh weight of edible part with shell length of mussels; linear regression equation with regression coefficient (r) and significance of regression coefficient

Enhancement of eutrophication by an increase in freshwater nutrients (phosphates) is thought to increase arsenic (+V) by biological processes, metabolism of phytoplankton and decomposition of organic matter by bacteria (Sohrin et al. 1997).

The low regression coefficient between length of shell and arsenic concentration, as well as the highly variable arsenic concentrations in the winter mussel population, is distinctly different from results obtained during other seasons. The seasonal balance between acquisition and utilization of nutrients is lower during winter, and protein reserves are probably used for metabolic requirements (Hawkins and Bayne 1985; 1992). The body weight of mussels changes over an annual cycle according to the relative rate of somatic and gonad growth.

Metal/shell weight indices provide a reliable tool for estimating metal bioavailability in coastal zones. Therefore, increased total arsenic in the winter population of mussels could be described, at least partially, as a combination of the natural variations resulting from nutritional nourishment and increased nutrient inputs, which precede phytoplankton production, favouring arsenic bioaccumulation, rather than as a result of arsenic pollution. Bioaccumulation of arsenic through the food chain, as well as mussel growth, depends on environmental factors, particularly the amount of food ingested and its quality, which determine the efficiency of filter-feeding assimilation (Winter 1978; Griffiths and King 1979).

The total arsenic content in the tail muscles of the Norway lobster *N. norvegicus* ranged from 10.6 to 25.7 mg As /kg FW (mean 17.11±4.48 mg As/kg FW). Our results are in agreement with data on total arsenic levels in Mediterranean Norway lobster (45 mg As/kg DW, recalculated as 9 mg As/kg FW; Storelli and Marcotrigiano 2001) and for the crustacean *Squilla mantis* (19.0 to 20.3 mg/kg FW; Storelli and Marcotrigiano 2000). Linear regression analysis showed a relationship between body length and arsenic concentration in Norway lobster *N. norvegicus* muscle (Fig. 5).

The WHO (1981) recommends a maximal tolerable daily intake of 50 μg As/kg body weight (inorganic arsenic). Thus, for a 70-kg human a tolerable dose would be 3.5 mg As/day (US FDA 1993). Assuming an average of 10 g seafood per day along the Adriatic coast, and an arsenic concentration typical of Norway lobster muscle, the daily intake of arsenic from seafood is much less than the tolerable daily intake of 3.5 mg/day. Concentrations of inorganic arsenic determined by standard methods were negligible in relation to the limit of 1 mg/kg, which actually applies not to fish and shellfish but total arsenic in other foodstuffs (Valette-Silver et al. 1999). There is no cause for concern as regards human health, as most of the arsenic is thought to be present as organoarsenic compounds of low mammalian toxicity.

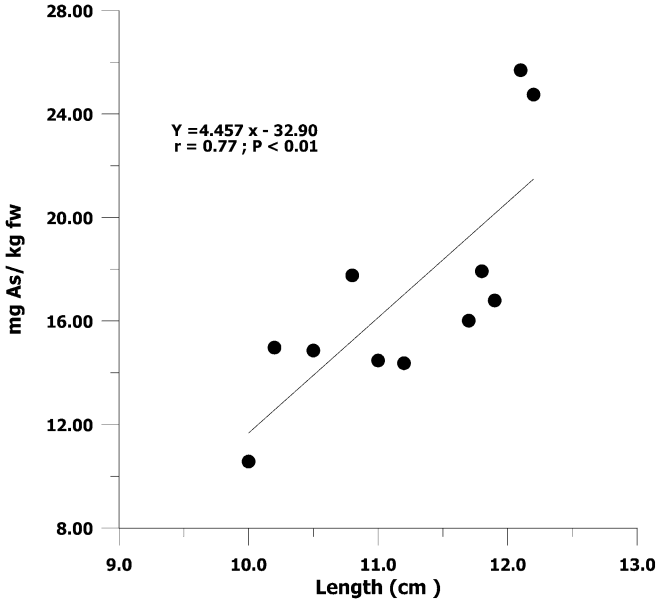

Fig. 5 Correlation between arsenic concentration in the tail muscle (mg As/kg FW) and length of the Norway lobsters *Nephrops norvegicus*; linear regression equation with regression coefficient (*r*) and significance of regression coefficient

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