



# Sex- and sexual maturation-related aspects of the element accumulation in soft tissues of the bivalve *Mytilus galloprovincialis* Lam. collected off coasts of Sevastopol (southwestern Crimea, Black Sea)

Sergey V. Kapranov<sup>1</sup> · Nadezhda V. Karavantseva<sup>1</sup> · Nikolay I. Bobko<sup>1</sup> · Vitaliy I. Ryabushko<sup>1</sup> · Larisa L. Kapranova<sup>1</sup> ·

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## Abstract

Mussels are widely used to monitor trace element pollution, but the role of their sex and sexual maturation in the element accumulation was not comprehensively studied and the number of elements under consideration never exceeded three dozen. In this work, concentrations of 67 elements in different tissues of females and males of the Mediterranean mussel are determined. Statistically significant differences are obtained in the element composition of analogous tissues of mussel males and females and in gonads of the same sex at different sexual maturation stages. The significantly higher concentrations of some elements found in gonads of females (Mn, Zn, As) and males (P) at most of the sexual maturation stages suggest essentiality of these elements in the reproduction. Elements exhibit several accumulation patterns in gonads during the ripening, with the most general one being the accumulation after spawning. Indications of a new phenomenon of circulation of most elements in gonads over the reproductive cycle are obtained, with the element concentrations decreasing at the first two stages and increasing at the posterior stages. Principal component analysis shows that the differences in the element accumulation are mainly due to the element uptake pathways and sexual differentiation. The most important identified factors determining the decrease in concentrations of tissue elements are their redistribution over mussel's body, biomass dilution, sequestration, and release with gametes. Five detected non-overlapping clusters of the non-normalized element concentrations in the pooled mussel tissues are associated with macroelements, two groups of trace elements, and two groups of ultratrace elements.

**Keywords** Mussels · *Mytilus galloprovincialis* · Gonadal ripening stages · Trace elements · ICP-MS · Accumulation · Multivariate analysis · Clusters

## Introduction

Mussels are known to be sentinel organisms for bioindication of coastal water pollution because of their remarkable ability to accumulate sea water pollutants (Adams and Rowland 2003; Rainbow and Phillips 1993). The “Mussel Watch” bio-monitoring programs implemented in several countries

include measuring concentrations of trace elements, petroleum hydrocarbons, and radioactive elements in mussels (Beliaeff et al. 1997; Farrington et al. 2016; Goldberg 1975; Goldberg and Bertine 2000). Several reviews on *Mytilus* spp. as indicators of heavy metal pollution have been published (Azizi et al. 2018; Beyer et al. 2017; Cossa 1989; Stankovic and Jovic 2012).

Many accumulated chemical elements are essential to biota so that the lack of an essential element in an organism can stop its growth or reproduction (Mertz 1986). The major ions such as sodium, potassium, calcium, and magnesium also known as macronutrients or macroelements are incorporated in a variety of biophysical and biochemical processes and are thus essential to sustain life. Some other metals and metalloids present in trace amounts in organisms (essential trace elements, or micronutrients) are also necessary for maintaining their

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✉ Sergey V. Kapranov  
sergey.v.kapranov@yandex.ru

<sup>1</sup> A.O. Kovalevsky Institute of Biology of the Southern Seas Russian Academy of Sciences Nakhimov ave. 2 Sevastopol 299011 Russian Federation

normal physiological functioning. Trace elements as enzyme cofactors form coordination complexes with proteins that have diverse biochemical functions (Bowen 1988). The borderline that delimits the areas of major and trace element concentrations in biological tissues is somewhat arbitrary. Different authors estimated it at the level of 100 ppm dry weight (Eisler 2010), 1 ppm wet weight (Valle 1952), or even 1% wet weight (Bowen 1988). International Union of Pure and Applied Chemistry defines trace elements as those with concentration less than 100 parts per million atoms or less than 100  $\mu\text{g}\cdot\text{g}^{-1}$  (McNaught and Wilkinson 1997).

Like many marine organisms, mussels have a potential to absorb both essential and non-essential elements in their tissues from the environment (Fowler 1990; Rainbow and Phillips 1993). The accumulation of elements raises their concentrations in soft tissues by several orders of magnitude above those in the surroundings (Casas et al. 2008). When accumulated in excess of the metabolic requirements, essential elements can be harmful for organisms (Rainbow 2002), and some non-essential metals—particularly, mercury, cadmium, and lead—exhibit very high toxicity at relatively small concentrations (Bat et al. 1999; Çevik et al. 2008) and are likely to affect normal functioning, e.g., fertilization, even in sub-toxic doses (De Guglielmo et al. 2019). However, marine organisms are capable of detoxifying the accumulated elements by sequestering metals on metal-binding sites (Giguère et al. 2003; Marigómez et al. 2002; Richir and Gobert 2016) in the particulate fraction of cells—granules and lysosomes (George 1990)—and in the cytosol—in metallothionein and metallothionein-like proteins (Amiard et al. 2006; George 1990; Shariati and Shariati 2011). Excessive amounts of trace elements can eventually be eliminated with feces and urine (Varotto et al. 2013).

Trace element biomonitoring using mussels aims to detect long-term trace element pollution and effects on native mussels, but it cannot help assess short-term toxic effects of trace elements on these organisms. In recent works, a problem of quick and effective toxicological assessment of heavy metal effects on mussels was addressed. The authors proposed male mussels' reproductive system and some other organs as a target and studied the effects of several heavy metals in sub-toxic concentrations on expression genes for several biomarkers: glutathione *S*-transferase  $\pi$ -gst (Piscopo et al. 2016), heat shock protein hsp70 (Piscopo et al. 2018a), metallothioneins *mt10* (Lettieri et al. 2019), and *mt20* (Piscopo et al. 2018b) as well as on properties of sperm chromatin protamine-like proteins (PL). The accumulation of most metals, except for Cd (De Guglielmo et al. 2019; Piscopo et al. 2018b), in tissues and levels of gene expression demonstrated complex dose-dependent relationships (Piscopo et al. 2016). The effects of Cd and Cu exposure turned out to be most univocal (Lettieri et al. 2019; Piscopo et al. 2018a), but PL of mussels sampled in summer, unlike winter, demonstrated no response to

cadmium (Piscopo 2019), which impugns the validity of using mussel PL as biomarkers of subtoxic heavy metal exposure.

The biological factors affecting the trace element accumulation within a single mussel species include age, size, sex, genotype, phenotype, feeding activity, and sexual maturity (Boening 1999; Mubiana et al. 2006; Richir and Gobert 2014; Saavedra et al. 2004). Interspecific differences in trace element bioaccumulation in mussels appear less significant than its geographic variation within a particular species (Blackmore and Wang 2003).

A large number of works addressing the trace element accumulation in mussels from different geographic areas were published, and the corresponding data were compiled (e.g., in Eisler 2010, Krishnakumar et al. 2018). In the Black Sea region, bioaccumulation of several trace elements in mussels *Mytilus galloprovincialis* living off coasts of Turkey (Bat et al. 1999; Çevik et al. 2008; Çulha et al. 2017; Tepe and Süer 2016), Romania (Roméo et al. 2005), the Kuban region (Temerdashev et al. 2017), and Crimea (Kozintsev 2006) was reported. However, the influence of biological factors on accumulation of trace elements in the Black Sea mussels was not studied in these works.

The studies of the sex-related differences in trace element concentrations in soft tissues of mussels started already in 1970s (Watling and Watling 1976) and continued in 1980–1990s (Latouche and Mix 1982; Lobel et al. 1991; Orren et al. 1980). It was noted that concentrations of some elements, e.g., manganese and zinc, were significantly higher in mollusk females, at least before spawning, but it was not reported to which tissue(s) this difference was related. In the same decades, the effects of seasonality on the trace element accumulation were studied (Fowler and Oregioni 1976; Lobel et al. 1991; Phillips 1976; Popham and D'Auria 1983; Simpson 1979) and they were interpreted as the effects of the reproductive cycle and body growth stages or climatologic peculiarities (increase in rainfall). The reproductive cycle stages were classified only as those before and after spawning, without the finer division into sexual maturation stages, which could be important in the element accumulation characterization.

In some recent studies, higher concentrations of trace elements in soft tissues and germ cells of mussels were registered in winter (Bajc and Kirbiš 2019; Giarratano and Amin 2010; Piscopo 2019). This feature was commonly explained by the slower growth of mollusks in the lower temperature and lower food availability conditions. Other factors, such as higher terrestrial water discharges carrying metals into the sea and varying biogeochemistry and speciation of metals, can also contribute to the winter accumulation of trace elements. In general, data on the metal-specific accumulation in different seasons vary (Bajc and Kirbiš 2019; Çulha et al. 2017; Giarratano and Amin 2010). However, opposite results were obtained in the work of Abderrahmani et al. (2020), in which the effect of seasonality on the metal accumulation was found

insignificant. The seasonality effect seems to be outweighed by, or at most to be equal in strength to, the effect of sampling station location within a range of some tens of km or closer (Abderrahmani et al. 2020; Phillips 1976; Rouane-Hacene et al. 2015). Generally, there is an obvious need for studying synergistic effects of multiple factors and stressors (Nardi et al. 2018) in the accumulation of trace elements, but this task presents serious methodological difficulties, not in the least due to biological response nonlinearity.

Many works have been focused on determining concentrations of only a limited number (typically, 4–8) of elements in mussel tissues. On the one hand, this limitation may be attributed to the instrumental difficulties of analysis because the main technique of the element quantification until recently was atomic absorption spectrometry (AAS), which usually allows measuring concentration of only one element per scan and is characterized by relatively high limits of detection. On the other hand, the interest in the trace element composition of mollusks is spurred mainly by environmental toxicity issues and the number of most toxic elements present in appreciable amounts in mollusk tissues is no greater than about a dozen.

The trace element analysis using inductively coupled plasma mass spectrometry (ICP-MS) is becoming increasingly popular due to numerous advantages of this method over the traditional AAS. In particular, it is much more sensitive and allows measuring concentrations of all known stable isotopes within a single scan of the instrument. Another valuable utility implemented in the modern ICP-MS is an option of *semiquantitative analysis* (Chen et al. 2008; Krzciuk 2016; Neubauer and Thompson 2011), in which concentrations of selected isotopes are estimated using the isotope ion signal and available calibration curves for analyte elements. In this case, the quantitation error is usually less than 50% (Chen et al. 2008; Krzciuk 2016).

The estimation of concentrations and distribution of as many elements as possible not only is needed for the assessment of environment pollution and determination of bioaccumulation features and pathways, but it also provides the primary information for biogeochemical studies. Methods of multivariate statistics (Rencher and Christensen 2012) help summarize effects of multiple factors in a system and thus can be used to explore general patterns of trace element bioaccumulation in different tissues of the mollusks. Multivariate analysis has been introduced to account for geographical (Abderrahmani et al. 2020; Bajt et al. 2019; Besada et al. 2011; Richir and Gobert 2014; Roméo et al. 2005; Struck et al. 1997; Szefer et al. 2006) or body compartmental (Richir and Gobert 2014) differences in the distribution of heavy metals in mussels, but it has not been applied to a suite of bioaccumulated elements assorted according to their abundance and grouping in mussel tissues. Taking into account possible important environmental, physiological

and biogeochemical implications of this information, the present work aims to fill this gap as applied to *M. galloprovincialis* living in coastal waters of the Sevastopol area (southwestern Crimea).

Other aims of this work are as follows: elucidating peculiarities of distribution of 67 trace elements in main tissues of Mediterranean mussels of both sexes in the same sampling period and area, with a special emphasis on female and male gonads at different sexual maturation stages and on general patterns of the trace element accumulation in them; revealing sex-related differences in the trace element composition of mussels' organs; and proposing a qualitative explanation of these peculiarities. This information, in its entirety, is completely novel and essential for better understanding molecular physiology of mussels and improving currently existing procedures of coastal pollution biomonitoring using mussels.

## Materials and methods

### Sample preparation

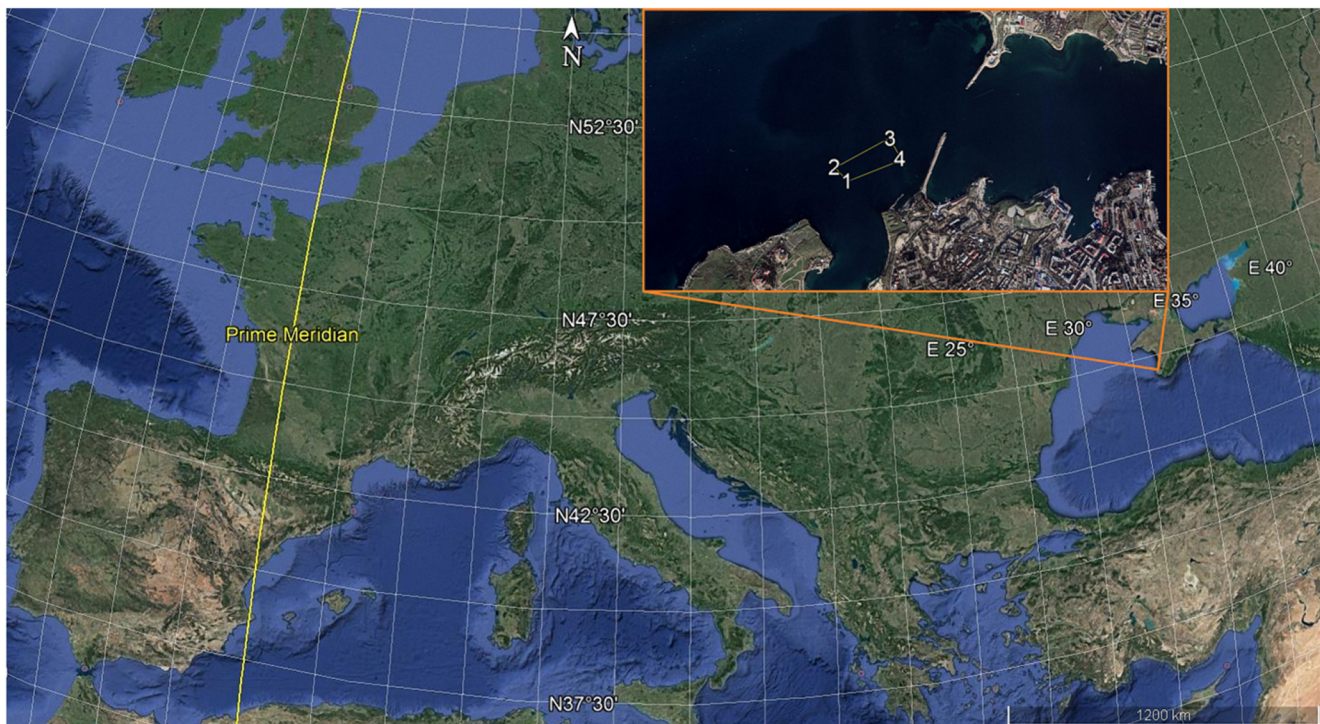
Mussels *M. galloprovincialis* were collected during their mass spawning in April–May 2017 at a depth of 6 m from the collectors of the mussel farm situated in the Black Sea (44° 61' 83.46" N, 33° 50' 33.80" E, Fig. 1) abeam the laboratory building of Institute of Biology of the Southern Seas in Sevastopol. The sea water temperature in the period of collecting was 9.8–11.0 °C. The shell length of the 2-year-old collected mussels varied in the range 60–80 mm. The sex of the mollusks and their sexual maturation on the six-stage scale were determined by examining gonadal smears under a microscope MBI-6 (Pirkova et al. 1994).

All the mussel shells were cleared from the incrustations and the mollusks were thoroughly washed in clean filtered seawater without being kept in the water for a long time for clearance of digestive tract since this could have led to inaccurate results because of the partial elimination of elements from soft tissues (Boening 1999).

Mussel tissues (with byssus excluded) were removed out of shells using plastic scalpel and tweezers, dissected, and blotted with filter paper that had been preliminarily rinsed in deionized water and dried. Samples of soft tissues (10–50 mg) were dried to constant weight in an oven at 105 °C and transferred by means of a plastic spatula into wet-ashing tubes made of PTFE.

Tissues of the following organs of mussel males and females were analyzed for trace elements: gills, foot, mantle edge, digestive system and heart, adductor muscle, and gonads at stages 3, 4, 5A, and 5B of sexual maturation. The trace elements were also determined in the male gonads at sexual





**Fig. 1** Mussel farm (quadrangle 1–2–3–4) off coasts of Sevastopol (Crimea) in the inset against the map of Europe

maturation stage 2 (females at the corresponding gonadal maturation stage had not been found).

The following sexual maturation (ripening) stages are distinguished according to qualitative changes in the gonads and germ cells of mussels (Pirkova et al. 1994): (1) resting, no visible germ cells; (2) gametogenesis onset, discernible oogonia, and spermatogonia; (3) active gametogenesis, growing oocytes, and spermatocytes; (4) pre-spawning, ripe oocytes, and spermatids separating from the walls of acini; (5) spawning, gametes traveling from acini through the genital ducts; and (6) post-spawning restructuring of gonads. In this work, two substages of the spawning stage 5 are discriminated: 5A (before the release of gametes in seawater) and 5B (immediately after the release of gametes). A similar gonad ripening classification was proposed in other works (McFarland et al. 2016; Seed 1969).

The digestion of the tissue samples was performed using 1.00 mL of 65% nitric acid of analytical grade additionally purified by double sub-boiling distillation in an acid purification system DST-1000 (Savillex, USA). Prior to the use in the sample preparations, all tubes had been decontaminated by immersion in this ultrapure nitric acid for about 2 days. The PTFE-capped wet-ashing tubes containing the samples and acid aliquots were kept in an autoclave at 120 °C for at least 2 h. The digested samples and all other solutions were diluted with deionized water with resistivity 18.2 M $\Omega$ ·cm purified in deionizer D-301 (Akvilon, Russia). The overall dilution factor for the samples was 2000 mL·g<sup>-1</sup> dry weight (dw).

### ICP-MS analysis

The element analysis was performed on a single-quadrupole ICP-MS instrument PlasmaQuant® MS Elite (Analytik Jena, Germany) fully controlled via PC. The approximate limits of detection of elements in the ICP-MS method vary from 0.1  $\mu\text{g L}^{-1}$  in aqueous solutions and 2 ppb in tissues (e.g., Rb, Cs, In, Tl, precious metals, and lanthanides) to 50  $\mu\text{g L}^{-1}$  in solutions and 1 ppm in tissues (K, Ca) (van de Wiel 2003).

The calibration curves were obtained using multielement standards IV-ICPMS-71A-C (Inorganic Ventures, USA) diluted with deionized water. Mixed standard solutions of lithium and mercury (II) nitrates (Supelco, USA) were used as separate standards. For the macroelements (Mg, Na, Al, Si, P, K, Ca), iron, bromine, and iodine, separate mixed standard solutions of higher concentrations were prepared using Mg(HCOO)<sub>2</sub>·2H<sub>2</sub>O, Ca(HCOO)<sub>2</sub>, KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O, (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, KH<sub>2</sub>PO<sub>4</sub>, (NH<sub>4</sub>)<sub>2</sub>SiF<sub>6</sub>, NaCl, KBrO<sub>3</sub>, and KIO<sub>3</sub> of analytical grade (Sigma-Aldrich) in 3% solution of extra pure nitric acid. *R*<sup>2</sup> coefficients of the linear fits for all the calibration curves were greater than 0.998.

The general parameters of the ICP-MS instrument and scan settings applied are given in Table S1 (Supplementary Material). The analyses were carried out with the collision reaction interface (CRI) switched off or on. In the CRI mode on, gaseous hydrogen with the flow rate 40 mL·min<sup>-1</sup> was used as the skimmer gas. CRI eliminates most of polyatomic interferences at the expense of the sensitivity and thus

enhances linearity of calibration curves and quantitation accuracy where these interferences are involved. Among 67 elements analyzed, 23 ones (Li, Be, B, Si, P, Ti, Co, Ni, Cu, Zn, As, Br, Mo, Cd, Sn, Sb, I, Ba, La, W, Ir, Pt, Tl) were quantified with CRI switched on because the corresponding  $R^2$  coefficients were higher in this mode.

To eliminate the signal drift and matrix effects (Agatemor and Beauchemin 2011; Ossipov et al. 2016), isotopes of six elements with concentrations  $20 \mu\text{g}\cdot\text{L}^{-1}$  were used as internal standards:  $^6\text{Li}$ ,  $^{45}\text{Sc}$ ,  $^{89}\text{Y}$ ,  $^{113,115}\text{In}$ ,  $^{159}\text{Tb}$ ,  $^{209}\text{Bi}$  (Bruker Daltonics Chemical Analysis, USA), given that in the preliminary analyses of the samples, these elements exhibited approximately constant levels that were close to zero.

The accuracy of the ICP-MS analysis was verified by the measurement of the element concentrations in the certified European Reference Material ERM®-CE278k (tissue of mussel *Mytilus edulis*,  $n = 5$ ) from Institute for Reference Materials and Measurements (Geel, Belgium). Samples of the certified reference material (0.1 g) were digested in the extra pure nitric acid, and the resulting solutions were diluted with deionized water to 10 mL according to the procedure described above. The mean element recoveries and uncertainties at the 95% confidence level along with the certified values are given in Table S2 (Supplementary Material).

For the calculation of bioconcentration factors, the concentrations of dissolved elements were obtained from the ICP-MS analysis of seawater filtered through 0.45- $\mu\text{m}$  membrane filters and diluted tenfold with deionized water so as to minimize the macroelement matrix effects. For the trace elements with questionable concentrations in the diluted seawater, we adopted the values reported elsewhere: in Ryabushko et al. (2017) for Si and P; in Egorov et al. (2018) for Cu, Zn, Cd, Pb, and Hg; in Colodner et al. (1995) for Re and U; and in German et al. (1991) and Ryabinin et al. (2011) for lanthanides.

## Statistical comparisons

The statistical significance of the element concentration difference in analogous tissues and between gonadal and somatic tissues of male and female mussels is estimated using the nonparametric Kolmogorov-Smirnov test for two samples and the Wilcoxon rank sum test (Hollander and Wolfe 1999) realized in the Matlab Statistics Toolbox functions *kstest2* and *ranksum*, respectively. The null hypothesis of the identity of samples is rejected only if it is rejected by both tests at the significance level  $p = 0.05$ .

The element concentrations in gonads at different stages of the reproductive cycle are compared using either of the two methods: if the Welch ANOVA realized in the Matlab function *wanova* (Penn 2009) demonstrates lack of homogeneity of variances at  $p = 0.05$ , the Games-Howell pairwise test is applied (Mégevand 2017; Zar 2009); otherwise, the Kruskal-

Wallis test (Matlab Statistics Toolbox function *kruskalwallis*) followed by the post hoc Dunn's test (Dunn 1964) at  $p = 0.05$  is used.

The significance of differences of the normalized concentration increments in a pair of sexual maturation stages is assessed using an original distribution analysis, which is described in detail in the “Results and discussion” section. When comparing the overall tendencies of the element concentrations in gonads as paired populations at two stages of the reproductive cycle, the one-sided Wilcoxon signed rank test realized as the Matlab Statistics Toolbox function *signrank* is applied to detect positive, negative, or zero increments ( $p = 0.05$ ).

## Multivariate statistics: principal component analysis and cluster analysis

### Principal component analysis

Principal component analysis seeks, using orthogonal transformation, the standardized linear combination of the original variables which has maximal variance (Mardia et al. 1979; Rencher and Christensen 2012). This method allows reducing the total number of variables to a few linear combinations of most significant—in terms of the resulting variance—ones. In the present work, the function *pca* embedded in Matlab Statistics Toolbox is applied, with employing the alternating least squares algorithm, maximum number of 3000 iterations, and default tolerances ( $10^{-6}$ ). The most important output arguments of this function are as follows: (1) a matrix of principal component coefficients also known as loadings, (2) a matrix of principal component scores, (3) a vector of principal component variances that are eigenvalues of the covariance matrix of the input data, and (4) a vector of percentage of the total variance explained by each principal component.

The normalized principal component coefficients for each variable and the principal component scores for each observation are often visualized in a single plot (biplot) as vectors and points, respectively. The direction and length of the vectors show how much the variables contribute to the principal component in the plot.

### Cluster analysis and optimal clustering evaluation

In this work, we use two clustering algorithms, the hierarchical and centroid-based clustering, and apply them for finding clusters of trace element concentrations in tissues. These approaches allow identifying families of elements with comparable concentrations due to either their hydrogeological abundance or bioaccumulation. Other possible clustering strategies, such as the distribution-based and density-based clustering, are more complex for realization and interpretation. They



are not expected to add any qualitatively new information to the results and are not used in this study.

In Matlab Statistics Toolbox, the agglomerative hierarchical clustering is realized in *linkage* function. The Euclidean distance between pairs of groups is used in this work as distance metrics and Ward's method as an approach for choosing a pair of clusters to merge at each iteration step.

As a centroid-based clustering algorithm, the Matlab function *kmeans* is applied in this study. The values of *k* are set to range from 1 to 11, and the squared Euclidean metric and other default arguments are used to find distances to cluster centroids. To ensure finding of the global minima in the sums of distances, the function is run  $2 \cdot 10^6$  times.

The optimal number of clusters for the centroid-based clustering results is sought in this work using two methods: the minimum statistical energy and the bend of the logarithmic distance sums on the *k* scale. The minimum statistical energy test (Aslan and Zech 2005; Székely and Rizzo 2013) coded in Matlab is available in open access (Lau 2014) and is used in this work.

Another method of finding optimal non-overlapping clusters is using the sum of within-cluster sums of logarithms of distances from centroids:

$$\Upsilon(k) = \sum_{i=1}^k \sum_{x_i \in C_i} \ln d(x_i, c_i) \quad (1)$$

where  $x_i$  and  $c_i$  denote objects and centroid of a cluster  $C_i$ , respectively, and  $d(x_i, c_i)$  is the distance between them obtained using *kmeans* function.

The optimal number of clusters is the integer closest to the elbow of this function, or in geometrical terms, to the intersection point of two linear fits for which the sum of their mean squared residuals is smallest. The linear fitting is justified by the uniform enough distribution of objects within clusters (Kapranov et al. 2013). Once the number *k* of clusters becomes greater than optimal, the line break occurs, with the slope of the posterior fit line being smaller in magnitude.

More information on the cluster analysis used in this work is in [Supplementary Material](#).

## Results and discussion

### Trace element bioaccumulation

The measured element concentrations in the soft tissues of female and male mussels per dry weight are summarized in Tables 1 and 2, respectively. One can notice the considerable enrichment in such elements as B, K, Au, and perhaps Ti, Ga, and La as compared with their ordinary levels in mollusks (Çevik et al. 2008; Eisler 2010) and the simultaneous depletion in calcium. The high concentrations of potassium and boron in the seawater are likely due to the riverine runoff

carrying potassium fertilizers and sewage discharge containing borax, a component of many detergent formulations. Lanthanum and other lanthanides can be transported with the riverine runoff because they are frequently used in agriculture as additives to fertilizers (Redling 2006), but it is also likely that this pollutant can be introduced in the form of suspension of fluorescent phosphors (Kujawski and Pospiech 2014). The increased concentrations of titanium may be a result of the wastewater discharge from the titania factory in the north of Crimea. The enrichment of the mussel tissues in gold and gallium is more difficult to explain.

In the collective accumulation of trace elements in the mussels from the Sevastopol area, one can expect also the existence of the synergistic and antagonistic effects (Azizi et al. 2018; Elliott et al. 1986; Jackim et al. 1977), which are still insufficiently studied and poorly understood.

Lanthanum was shown to bind to  $\text{Ca}^{2+}$ -binding sites, with inhibiting Ca-channels in cell membranes, interacting with membrane enzymes, and affecting tissue structure (Herrmann et al. 2016). This element was readily accumulated as inclusions of insoluble lanthanum phosphate in calcium-enriched granules in lysosomes, macrophage hemocytes, and chitinous tissue of *M. edulis* (Chassard-Bouchard and Hallégot 1984). The La:Ca ratio in these mussels varied from  $5 \cdot 10^{-4}$  to  $2 \cdot 10^{-3}$ , being comparable with our results.

Among toxic heavy metals, relatively high levels of mercury, about  $1 \text{ mg} \cdot \text{kg}^{-1} \text{ dw}$ , are registered in the mussel tissues, but this value is still smaller than the maximum permissible level established in the European Union regulations,  $0.5 \text{ mg} \cdot \text{kg}^{-1} \text{ w.w.}$  (Anonymous 2006) corresponding to approximately  $3 \text{ mg} \cdot \text{kg}^{-1} \text{ dw}$ . The found values are in close agreement with those reported earlier (Ryabushko et al. 2002; Ryabushko et al. 2005) for mussels collected in coastal waters of the southern and western Crimea, indicating that no dramatic rise or decline in mercury concentration in the coastal waters has occurred since 1984.

Bioconcentration factors are the ratios of concentrations of a chemical in tissue and in water (Adams and Rowland 2003), and they are convenient measures to visualize regularities and levels of element accumulation, but because of a large number of variables influencing the trace element accumulation in mussel tissues, the bioconcentration factors are not invariants and can vary in a broad range. The mean bioconcentration factors for the soft tissues of male and female mussels collected in our work are presented in Fig. 2.

When considering trace element bioaccumulation in mussels, it is necessary to bear in mind that a large fraction of trace elements, no less than 30%, is ingested with food rather than absorbed directly from water, and in the case of selenium, this fraction reaches as much as 98% (Wang and Fisher 1997). For this reason, the uptake of trace elements in mussels can be regarded primarily as the accumulation in the plankton–mussels food chain link. Bioconcentration as a process of

**Table 1** Concentration of elements in female mussel tissues calculated per dry weight: mean ± standard error. *Italic emphasis*, in ‰ (parts per thousand); **bold emphasis**, in ppm; no emphasis, in ppb. *G*, gills; *F*, foot; *M*, mantle edge, *D+H*, digestive system and heart; *A*, adductor muscle;

*G<sub>i</sub>*, gonads at the sexual maturation stage *i*. The number of samples is given in parentheses. Asterisk (\*): the concentrations were obtained semi-quantitatively.

	G (5)	F (5)	M (5)	D+H (5)	A (5)	G3 (10)	G4 (5)	G5A (10)	G5B (10)
Li	98 ± 7	101 ± 2	90 ± 3	92 ± 4	114 ± 20	104 ± 4	84 ± 2	89 ± 4	111 ± 6
Be	8.9 ± 3.7	19.5 ± 4.1	11.1 ± 6.3	11.2 ± 3.1	4.5 ± 2.5	20.0 ± 4.7	13.3 ± 9.6	18.9 ± 5.4	21.1 ± 5.5
<b>B</b>	<b>35 ± 7</b>	<b>40 ± 7</b>	<b>28 ± 5</b>	<b>26 ± 3</b>	<b>29 ± 6</b>	<b>24 ± 1</b>	<b>19 ± 2</b>	<b>24 ± 3</b>	<b>33 ± 4</b>
<b>F*</b>	<b>452 ± 56</b>	<b>429 ± 32</b>	<b>388 ± 47</b>	<b>398 ± 41</b>	<b>261 ± 57</b>	<b>585 ± 40</b>	<b>413 ± 43</b>	<b>420 ± 54</b>	<b>741 ± 30</b>
<i>Na</i>	8.6 ± 0.5	11.4 ± 0.3	11.1 ± 0.5	10.8 ± 1.1	11.1 ± 1.3	17.7 ± 1.0	11.0 ± 1.4	10.9 ± 1.2	20.2 ± 0.8
<i>Mg</i>	4.6 ± 1.4	4.1 ± 0.6	4.2 ± 0.9	3.3 ± 0.8	5.0 ± 1.3	4.0 ± 0.3	2.6 ± 0.3	4.5 ± 0.7	5.4 ± 1.0
<i>Al</i>	2.1 ± 1.0	1.5 ± 0.2	1.3 ± 0.7	4.8 ± 3.1	8.3 ± 4.0	0.52 ± 0.08	1.05 ± 0.14	4.1 ± 1.6	5.1 ± 1.9
<i>Si</i>	1.9 ± 0.9	1.8 ± 0.7	1.1 ± 0.2	4.3 ± 2.6	0.9 ± 0.2	0.67 ± 0.05	1.0 ± 0.3	1.2 ± 0.3	0.74 ± 0.09
<i>P</i>	5.6 ± 0.5	5.4 ± 0.4	4.4 ± 0.4	5.9 ± 0.3	7.5 ± 1.7	6.7 ± 0.4	6.4 ± 0.5	4.4 ± 0.5	6.3 ± 0.3
<i>K</i>	12.8 ± 1.2	12.9 ± 0.4	11.6 ± 1.1	11.8 ± 0.7	13.4 ± 1.4	13.5 ± 0.8	10.2 ± 1.1	10.8 ± 1.0	13.9 ± 0.5
<i>Ca</i>	2.3 ± 0.5	2.5 ± 0.3	2.2 ± 0.4	2.7 ± 0.7	2.3 ± 0.7	1.42 ± 0.06	1.34 ± 0.23	5.9 ± 3.7	1.9 ± 0.3
<b>Ti</b>	<b>171 ± 53</b>	<b>182 ± 56</b>	<b>180 ± 55</b>	<b>240 ± 84</b>	<b>198 ± 56</b>	<b>85 ± 9</b>	<b>276 ± 187</b>	<b>161 ± 41</b>	<b>151 ± 22</b>
<b>V</b>	<b>1.41 ± 0.41</b>	<b>1.53 ± 0.64</b>	<b>1.05 ± 0.19</b>	<b>1.93 ± 0.71</b>	<b>0.89 ± 0.10</b>	<b>0.65 ± 0.02</b>	<b>1.21 ± 0.45</b>	<b>0.80 ± 0.09</b>	<b>0.77 ± 0.03</b>
<b>Cr</b>	<b>2.54 ± 0.21</b>	<b>2.99 ± 0.16</b>	<b>4.00 ± 1.00</b>	<b>3.26 ± 0.50</b>	<b>2.69 ± 0.03</b>	<b>2.71 ± 0.15</b>	<b>6.36 ± 3.45</b>	<b>3.18 ± 0.51</b>	<b>2.88 ± 0.26</b>
<b>Mn</b>	<b>7.37 ± 1.73</b>	<b>7.31 ± 1.90</b>	<b>4.70 ± 1.11</b>	<b>10.70 ± 2.28</b>	<b>6.52 ± 1.98</b>	<b>7.37 ± 1.74</b>	<b>7.56 ± 2.24</b>	<b>11.4 ± 1.6</b>	<b>11.7 ± 1.31</b>
<b>Fe</b>	<b>216 ± 91</b>	<b>208 ± 118</b>	<b>78 ± 14</b>	<b>380 ± 205</b>	<b>86 ± 14</b>	<b>87 ± 12</b>	<b>76 ± 26</b>	<b>70 ± 14</b>	<b>104 ± 19</b>
Co	864 ± 406	882 ± 641	70 ± 8	1098 ± 577	180 ± 71	55 ± 7	46 ± 14	35 ± 4	66 ± 6
<b>Ni</b>	<b>2.2 ± 1.0</b>	<b>2.1 ± 1.3</b>	<b>0.8 ± 0.2</b>	<b>2.6 ± 1.3</b>	<b>0.4 ± 0.1</b>	<b>0.8 ± 0.4</b>	<b>0.6 ± 0.2</b>	<b>0.4 ± 0.1</b>	<b>0.4 ± 0.1</b>
<b>Cu</b>	<b>3.6 ± 0.6</b>	<b>4.3 ± 1.0</b>	<b>2.4 ± 0.3</b>	<b>3.5 ± 0.7</b>	<b>2.8 ± 0.5</b>	<b>3.1 ± 0.4</b>	<b>2.7 ± 0.6</b>	<b>3.1 ± 0.6</b>	<b>4.4 ± 0.3</b>
<b>Zn</b>	<b>48 ± 15</b>	<b>72 ± 39</b>	<b>39 ± 11</b>	<b>70 ± 20</b>	<b>60 ± 14</b>	<b>36 ± 5</b>	<b>45 ± 11</b>	<b>56 ± 8</b>	<b>54 ± 5</b>
<b>Ga</b>	<b>0.44 ± 0.12</b>	<b>0.35 ± 0.05</b>	<b>1.3 ± 0.9</b>	<b>0.8 ± 0.4</b>	<b>1.4 ± 0.6</b>	<b>0.24 ± 0.01</b>	<b>1.9 ± 1.4</b>	<b>0.73 ± 0.24</b>	<b>0.77 ± 0.20</b>
Ge	61.2 ± 2.4	57.3 ± 5.4	52.0 ± 2.3	63.0 ± 8.0	52.5 ± 3.7	49.9 ± 1.5	52.0 ± 2.6	49.7 ± 1.5	54.7 ± 0.9
<b>As</b>	<b>23.8 ± 3.8</b>	<b>24.0 ± 3.6</b>	<b>13.1 ± 2.5</b>	<b>23.4 ± 3.3</b>	<b>20.0 ± 4.3</b>	<b>20.8 ± 1.9</b>	<b>11.2 ± 1.7</b>	<b>40.0 ± 10.3</b>	<b>22.9 ± 1.1</b>
<b>Se</b>	<b>18.1 ± 6.3</b>	<b>16.0 ± 1.0</b>	<b>13.2 ± 2.1</b>	<b>18.5 ± 5.3</b>	<b>23.0 ± 8.4</b>	<b>10.8 ± 0.8</b>	<b>9.0 ± 0.9</b>	<b>9.7 ± 1.5</b>	<b>13.7 ± 0.8</b>
<b>Br</b>	<b>83 ± 20</b>	<b>103 ± 26</b>	<b>65 ± 17</b>	<b>66 ± 12</b>	<b>61 ± 16</b>	<b>48 ± 3</b>	<b>36 ± 4</b>	<b>209 ± 98</b>	<b>65 ± 8</b>
<b>Rb</b>	<b>3.9 ± 0.3</b>	<b>4.0 ± 0.3</b>	<b>3.2 ± 0.3</b>	<b>3.7 ± 0.2</b>	<b>3.9 ± 0.5</b>	<b>3.9 ± 0.3</b>	<b>2.8 ± 0.3</b>	<b>3.1 ± 0.3</b>	<b>4.0 ± 0.3</b>
<b>Sr</b>	<b>48 ± 12</b>	<b>46 ± 5</b>	<b>43 ± 10</b>	<b>44 ± 8</b>	<b>48 ± 15</b>	<b>33.6 ± 1.4</b>	<b>23 ± 4</b>	<b>68 ± 28</b>	<b>44 ± 9</b>
Zr	14 ± 5	23 ± 6	11 ± 3	18 ± 10	16 ± 12	8 ± 2	54 ± 39	15 ± 5	37 ± 8
Nb	38 ± 17	57 ± 21	16 ± 5	67 ± 35	15 ± 4	7 ± 1	21 ± 11	16 ± 7	28 ± 5
Mo	161 ± 57	183 ± 87	57 ± 19	169 ± 58	65 ± 18	58 ± 4	130 ± 85	41 ± 6	72 ± 15
Ru	5.1 ± 1.2	3.2 ± 0.5	3.0 ± 0.3	3.8 ± 0.7	4.4 ± 0.8	5.0 ± 0.7	4.1 ± 1.4	10.7 ± 4.1	25.1 ± 2.4
Rh	44 ± 5	73 ± 19	71 ± 8	37 ± 6	43 ± 6	53 ± 4	58 ± 12	66 ± 14	35 ± 3
Pd	709 ± 22	773 ± 42	713 ± 30	659 ± 23	773 ± 118	751 ± 22	713 ± 29	705 ± 20	813 ± 40
<b>Ag</b>	<b>0.85 ± 0.25</b>	<b>1.52 ± 0.83</b>	<b>0.73 ± 0.18</b>	<b>0.48 ± 0.04</b>	<b>0.53 ± 0.06</b>	<b>0.55 ± 0.01</b>	<b>0.67 ± 0.12</b>	<b>0.63 ± 0.03</b>	<b>0.62 ± 0.03</b>
<b>Cd</b>	<b>2.00 ± 0.95</b>	<b>2.11 ± 1.32</b>	<b>0.56 ± 0.11</b>	<b>1.62 ± 0.66</b>	<b>0.60 ± 0.19</b>	<b>1.23 ± 0.09</b>	<b>1.59 ± 1.03</b>	<b>0.58 ± 0.13</b>	<b>0.84 ± 0.11</b>
Sn	109 ± 44	267 ± 91	146 ± 25	97 ± 27	83 ± 42	251 ± 79	221 ± 61	485 ± 196	483 ± 63
Sb	130 ± 34	154 ± 40	78 ± 14	124 ± 35	45 ± 7	82 ± 9	89 ± 24	98 ± 24	67 ± 9
Te	167 ± 139	10.3 ± 3.4	7.2 ± 0.4	11.7 ± 2.7	7.6 ± 1.3	10.7 ± 0.9	162 ± 140	8.5 ± 1.3	11.8 ± 0.8
<b>I</b>	<b>43 ± 5</b>	<b>37 ± 6</b>	<b>31 ± 6</b>	<b>51 ± 9</b>	<b>35 ± 6</b>	<b>14 ± 2</b>	<b>25 ± 3</b>	<b>48 ± 14</b>	<b>17 ± 2</b>
Cs	16 ± 3	121 ± 73	25 ± 6	17 ± 7	15 ± 5	34 ± 12	38 ± 18	64 ± 26	107 ± 36
<b>Ba</b>	<b>7.67 ± 3.41</b>	<b>6.24 ± 2.63</b>	<b>2.55 ± 0.84</b>	<b>12.1 ± 6.7</b>	<b>1.19 ± 0.32</b>	<b>1.20 ± 0.27</b>	<b>0.99 ± 0.23</b>	<b>2.61 ± 1.58</b>	<b>2.05 ± 0.44</b>
<b>La</b>	<b>1.17 ± 0.15</b>	<b>1.87 ± 0.45</b>	<b>1.66 ± 0.26</b>	<b>1.24 ± 0.45</b>	<b>1.65 ± 0.50</b>	<b>3.42 ± 0.45</b>	<b>1.70 ± 0.41</b>	<b>3.28 ± 1.16</b>	<b>4.04 ± 1.89</b>
Ce	469 ± 205	526 ± 258	160 ± 34	810 ± 468	196 ± 53	240 ± 65	169 ± 54	180 ± 36	227 ± 47
Pr	45 ± 15	44 ± 23	14 ± 3	67 ± 37	16 ± 4	16.9 ± 0.9	20 ± 4	12 ± 3	16 ± 2
Nd	249 ± 85	229 ± 123	71 ± 15	396 ± 215	86 ± 23	94 ± 5	130 ± 36	68 ± 15	88 ± 12
Sm	82 ± 35	39 ± 22	11.1 ± 3.0	66 ± 36	12 ± 3	13.6 ± 0.7	56 ± 38	10.3 ± 2.2	13.6 ± 1.9

**Table 1** (continued)

	G (5)	F (5)	M (5)	D+H (5)	A (5)	G3 (10)	G4 (5)	G5A (10)	G5B (10)
Eu	263 ± 82	133 ± 76	44 ± 14	268 ± 152	28 ± 8	35 ± 3	121 ± 79	50 ± 25	36 ± 5
Gd	55 ± 17	35 ± 20	11 ± 3	58 ± 31	10 ± 3	14.9 ± 0.9	31 ± 16	12 ± 2	13 ± 2
Dy	20.2 ± 0.4	21.0 ± 0.2	19.4 ± 0.5	19.4 ± 0.5	23 ± 4	19.7 ± 0.3	19.5 ± 0.4	19.1 ± 0.6	18.9 ± 0.7
Ho	10.4 ± 3.1	6.7 ± 3.9	2.2 ± 0.6	11.7 ± 5.9	2.6 ± 0.6	3.4 ± 0.2	5.8 ± 2.7	2.5 ± 0.4	3.0 ± 0.4
Er	65 ± 16	45 ± 19	23 ± 2	65 ± 26	29 ± 4	30.9 ± 1.4	42 ± 16	25.1 ± 2.3	27.8 ± 2.4
Tm	3.1 ± 0.9	2.3 ± 1.4	0.6 ± 0.2	3.9 ± 1.9	1.0 ± 0.3	1.3 ± 0.1	1.5 ± 0.6	0.9 ± 0.2	1.2 ± 0.2
Yb	23 ± 6	17 ± 9	6.2 ± 2.1	31 ± 14	7.7 ± 2.2	12.4 ± 0.7	12.4 ± 5.3	8.5 ± 1.3	10.8 ± 2.1
Lu	18 ± 5	16 ± 9	4.8 ± 1.1	25 ± 12	8.2 ± 2.3	11.2 ± 0.8	6.6 ± 1.5	7.6 ± 1.1	9.5 ± 1.5
Hf	169 ± 6	178 ± 5	167 ± 6	166 ± 3	196 ± 30	174 ± 4	171 ± 3	163 ± 5	173 ± 7
Ta	96 ± 73	20 ± 5	10.8 ± 1.6	16 ± 4	10.8 ± 1.6	8.1 ± 0.9	94 ± 74	9.9 ± 0.9	11.7 ± 1.3
W	285 ± 88	768 ± 231	516 ± 197	319 ± 66	159 ± 27	294 ± 123	359 ± 49	434 ± 100	836 ± 119
Re	18 ± 16	0.9 ± 0.1	1.6 ± 0.8	0.9 ± 0.1	0.6 ± 0.2	0.7 ± 0.1	18 ± 15	0.7 ± 0.0	0.7 ± 0.1
Os	1107 ± 948	42 ± 12	94 ± 47	45 ± 8	40 ± 7	32 ± 5	1091 ± 951	31 ± 5	42 ± 3
Ir	58 ± 10	82 ± 26	98 ± 13	35 ± 6	58 ± 10	56 ± 8	81 ± 26	49 ± 12	36 ± 8
Pt	55 ± 7	121 ± 46	104 ± 17	54 ± 13	59 ± 7	74 ± 7	92 ± 15	72 ± 10	36 ± 8
Au	306 ± 54	460 ± 167	191 ± 43	189 ± 42	171 ± 17	249 ± 117	225 ± 37	388 ± 112	204 ± 92
<b>Hg</b>	<b>1.06 ± 0.25</b>	<b>2.02 ± 0.91</b>	<b>0.61 ± 0.07</b>	<b>0.72 ± 0.13</b>	<b>0.62 ± 0.09</b>	<b>0.93 ± 0.45</b>	<b>0.86 ± 0.14</b>	<b>1.64 ± 0.51</b>	<b>0.97 ± 0.43</b>
Tl	16 ± 4	34 ± 11	15 ± 3	27 ± 10	13 ± 3	17 ± 10	14 ± 4	21 ± 11	36 ± 18
<b>Pb</b>	<b>3.72 ± 1.22</b>	<b>3.66 ± 1.91</b>	<b>1.39 ± 0.37</b>	<b>5.34 ± 1.82</b>	<b>4.73 ± 1.91</b>	<b>1.42 ± 0.17</b>	<b>2.79 ± 1.71</b>	<b>0.91 ± 0.13</b>	<b>1.90 ± 0.39</b>
Th	67 ± 33	189 ± 98	10.2 ± 2.7	78 ± 42	11.6 ± 2.1	60 ± 49	19 ± 4	254 ± 181	46 ± 30
U	184 ± 68	196 ± 89	69 ± 15	231 ± 104	52 ± 15	52 ± 8	76 ± 35	56 ± 17	57 ± 8

direct extraction of elements from water by mussels is of secondary importance, and in this respect, the term *bioconcentration factor* is only nominal. A multitude of underlying routes of the element uptake (i.e., both bioconcentration and bioaccumulation) aggregately contribute to its value.

The highest bioconcentration factors ( $10^5$ – $10^6$ ) are observed for phosphorus and some lanthanides (La, Sm, and Eu). Lanthanides have similar chemical properties and thus can be accumulated in the same pathways as lanthanum does, substituting essential elements, such as Ca, Mn, Fe, and Zn, due to the similarity of their ionic radii and coordination numbers. In contrast, phosphorus is an essential nutrient responsible for accumulation and release of energy in living cells, and it is extensively absorbed from the water column by plankton (Comer 1973). It is worthwhile to note that the uncertainty in determination of the bioconcentration factors can reach more than 100% of the mean value as shown by an example of phosphorus, silicon, and nickel (Fig. 2).

The lowest bioconcentration factors ( $10^0$ – $10^1$ ) are obtained for boron, sodium, magnesium, and germanium. In case of sodium and magnesium, this is explained by their high concentrations in seawater. The low bioconcentration factors of boron and germanium are associated with their low affinity to tissues and a seeming lack of any physiological role in mussels.

### Sex-related differences in the element bioaccumulation in the mussel tissues

The elements with significantly different concentrations in analogous tissues of male and female mussels, as detected in the Kolmogorov-Smirnov and Wilcoxon rank sum tests, are presented in Table 3. From these data only, it is not possible to determine the role of one or another element in the sexual differentiation in mussels, nor is it feasible to establish with which subcellular structure or biochemical process the higher concentrations of the elements are associated. For example, the significantly higher levels of Pb, Zn, and La in gills of male mussels may be due to sex-specific metal transportation in this organ. At the same time, this feature may originate from more intensive replacement of calcium or other essential ions by these elements or their deposition as insoluble carbonates in gills of males.

Female mussels accumulate greater amounts of many trace elements in gonads except for the early stage of sexual maturation, in overall agreement with the measurements in soft tissues in the pre-spawning period (Richir and Gobert 2016). Special attention should be paid to the elements which likely play an essential functional role in gametogenesis of mussels (Akberali et al. 1985; Latouche and Mix 1981). Female gonads differ from male ones in increased levels of As, Mn, and Zn at many sexual



**Table 2** Concentration of elements in male mussel tissues calculated per dry weight: mean ± standard error. The emphasis and notations are the same as in Table 1

	G (3)	F (3)	M (3)	D+H (3)	A (3)	G2 (10)	G3 (10)	G4 (3)	G5A (7)	G5B (10)
Li	103 ± 2	84 ± 3	102 ± 3	107 ± 13	87 ± 1	93 ± 13	108 ± 9	84 ± 4	95 ± 4	108 ± 1
Be	0	15 ± 3	7 ± 3	0	15 ± 8	11 ± 4	8 ± 4	15 ± 8	14 ± 5	20 ± 4
<b>B</b>	<b>38.2 ± 1.3</b>	<b>21.5 ± 2.6</b>	<b>45 ± 6</b>	<b>25.4 ± 2.3</b>	<b>17.7 ± 1.7</b>	<b>38 ± 12</b>	<b>20.7 ± 1.3</b>	<b>20.5 ± 0.9</b>	<b>26.7 ± 3.0</b>	<b>30.0 ± 2.7</b>
<b>F*</b>	<b>274 ± 36</b>	<b>279 ± 54</b>	<b>300 ± 42</b>	<b>324 ± 61</b>	<b>356 ± 42</b>	<b>426 ± 49</b>	<b>541 ± 41</b>	<b>328 ± 32</b>	<b>617 ± 35</b>	<b>529 ± 34</b>
Na	-	9.4 ± 0.6	-	8.7 ± 0.1	10.1 ± 0.9	13.1 ± 0.5	13.9 ± 1.0	10.0 ± 0.5	16.5 ± 1.1	16.9 ± 1.1
Mg	6.4 ± 0.1	2.7 ± 0.1	6.1 ± 0.5	2.2 ± 0.2	2.4 ± 0.2	5.9 ± 1.8	3.2 ± 0.2	2.4 ± 0.2	4.4 ± 0.5	4.0 ± 0.2
Al	1.5 ± 0.8	4.4 ± 1.1	1.3 ± 0.6	0.5 ± 0.1	0.6 ± 0.2	1.1 ± 0.4	1.4 ± 0.5	0.8 ± 0.2	1.5 ± 0.9	2.2 ± 0.6
Si	1.03 ± 0.30	2.0 ± 0.7	1.1 ± 0.3	3.5 ± 0.3	1.3 ± 0.3	1.04 ± 0.27	0.47 ± 0.03	0.87 ± 0.11	0.65 ± 0.04	0.89 ± 0.18
P	6.7 ± 0.1	5.0 ± 0.3	6.2 ± 1.4	6.9 ± 1.0	3.3 ± 0.2	16 ± 6	9.5 ± 0.8	6.9 ± 1.3	10.2 ± 1.2	16.8 ± 0.8
K	14.9 ± 0.3	13.5 ± 0.8	17.8 ± 3.1	13.0 ± 1.4	6.7 ± 0.1	11.2 ± 0.5	15.3 ± 1.0	14.0 ± 0.6	14.8 ± 0.8	17.5 ± 0.6
Ca	3.6 ± 0.8	1.3 ± 0.1	3.1 ± 0.3	2.1 ± 0.1	1.5 ± 0.1	2.7 ± 1.0	1.2 ± 0.1	1.3 ± 0.1	6.7 ± 4.3	1.5 ± 0.1
<b>Ti</b>	<b>172 ± 12</b>	<b>180 ± 16</b>	<b>161 ± 38</b>	<b>288 ± 67</b>	<b>81 ± 7</b>	<b>160 ± 63</b>	<b>76 ± 8</b>	<b>97 ± 24</b>	<b>80 ± 15</b>	<b>416 ± 160</b>
<b>V</b>	<b>0.91 ± 0.03</b>	<b>1.19 ± 0.25</b>	<b>1.17 ± 0.38</b>	<b>3.35 ± 0.63</b>	<b>0.66 ± 0.04</b>	<b>1.60 ± 0.52</b>	<b>0.68 ± 0.01</b>	<b>0.76 ± 0.09</b>	<b>0.75 ± 0.05</b>	<b>1.52 ± 0.37</b>
<b>Cr</b>	<b>3.3 ± 0.4</b>	<b>4.6 ± 1.3</b>	<b>4.9 ± 1.7</b>	<b>4.9 ± 1.4</b>	<b>2.4 ± 0.1</b>	<b>6.9 ± 2.5</b>	<b>2.5 ± 0.1</b>	<b>2.4 ± 0.1</b>	<b>2.6 ± 0.2</b>	<b>8.1 ± 2.3</b>
<b>Mn</b>	<b>10.7 ± 0.3</b>	<b>10.0 ± 1.0</b>	<b>3.9 ± 0.5</b>	<b>8.7 ± 1.3</b>	<b>1.9 ± 0.4</b>	<b>6.1 ± 2.2</b>	<b>2.8 ± 0.2</b>	<b>4.9 ± 2.1</b>	<b>2.6 ± 0.3</b>	<b>4.9 ± 1.4</b>
<b>Fe</b>	<b>104 ± 10</b>	<b>100 ± 17</b>	<b>80 ± 13</b>	<b>564 ± 38</b>	<b>32 ± 6</b>	<b>152 ± 46</b>	<b>74 ± 11</b>	<b>54 ± 11</b>	<b>95 ± 28</b>	<b>139 ± 28</b>
Co	331 ± 23	65 ± 12	64 ± 3	2190 ± 346	71 ± 34	49 ± 22	20 ± 4	18 ± 6	38 ± 16	37 ± 7
<b>Ni</b>	<b>0.90 ± 0.11</b>	<b>0.61 ± 0.14</b>	<b>0.64 ± 0.10</b>	<b>5.31 ± 0.79</b>	<b>0.23 ± 0.02</b>	<b>0.58 ± 0.23</b>	<b>0.29 ± 0.04</b>	<b>0.24 ± 0.04</b>	<b>0.27 ± 0.04</b>	<b>0.47 ± 0.10</b>
<b>Cu</b>	<b>4.20 ± 0.27</b>	<b>2.94 ± 0.39</b>	<b>2.88 ± 0.40</b>	<b>5.26 ± 0.88</b>	<b>0.83 ± 0.11</b>	<b>5.65 ± 2.16</b>	<b>2.18 ± 0.17</b>	<b>1.73 ± 0.52</b>	<b>1.74 ± 0.08</b>	<b>2.61 ± 0.25</b>
<b>Zn</b>	<b>151 ± 18</b>	<b>22 ± 1</b>	<b>27 ± 4</b>	<b>91 ± 18</b>	<b>58 ± 18</b>	<b>33 ± 8</b>	<b>24.6 ± 1.6</b>	<b>16.3 ± 1.5</b>	<b>22.9 ± 2.3</b>	<b>27.5 ± 1.6</b>
<b>Ga</b>	<b>0.42 ± 0.15</b>	<b>0.68 ± 0.13</b>	<b>0.32 ± 0.11</b>	<b>0.29 ± 0.02</b>	<b>0.19 ± 0.03</b>	<b>0.51 ± 0.16</b>	<b>0.43 ± 0.06</b>	<b>0.31 ± 0.01</b>	<b>0.48 ± 0.14</b>	<b>2.89 ± 1.12</b>
Ge	59.9 ± 0.6	52.6 ± 2.0	56 ± 3	70 ± 4	43.9 ± 1.2	62 ± 9	46.9 ± 0.7	47.3 ± 1.1	49.1 ± 2.2	50.3 ± 1.9
<b>As</b>	<b>32.1 ± 1.4</b>	<b>11.7 ± 0.3</b>	<b>21.7 ± 2.0</b>	<b>28 ± 4</b>	<b>7.8 ± 0.9</b>	<b>24 ± 6</b>	<b>14.3 ± 0.9</b>	<b>13.4 ± 0.9</b>	<b>17.8 ± 3.1</b>	<b>13.3 ± 1.5</b>
<b>Se</b>	<b>40.8 ± 2.2</b>	<b>16.9 ± 2.1</b>	<b>17.8 ± 0.4</b>	<b>13.4 ± 1.0</b>	<b>6.8 ± 0.6</b>	<b>12.0 ± 1.9</b>	<b>8.7 ± 0.3</b>	<b>9.3 ± 1.6</b>	<b>10.7 ± 1.1</b>	<b>8.8 ± 0.6</b>
<b>Br</b>	<b>88.1 ± 1.6</b>	<b>39.6 ± 1.7</b>	<b>93 ± 10</b>	<b>55 ± 4</b>	<b>32 ± 4</b>	<b>49 ± 12</b>	<b>38 ± 2</b>	<b>30 ± 6</b>	<b>60 ± 6</b>	<b>36 ± 4</b>
<b>Rb</b>	<b>4.18 ± 0.03</b>	<b>3.8 ± 0.3</b>	<b>5.1 ± 0.9</b>	<b>5.0 ± 0.5</b>	<b>1.86 ± 0.08</b>	<b>7.9 ± 3.0</b>	<b>4.6 ± 0.5</b>	<b>3.81 ± 0.12</b>	<b>4.0 ± 0.3</b>	<b>4.58 ± 0.24</b>
<b>Sr</b>	<b>71 ± 12</b>	<b>23.7 ± 0.7</b>	<b>62 ± 4</b>	<b>37 ± 3</b>	<b>25.8 ± 2.4</b>	<b>52 ± 16</b>	<b>25.0 ± 2.0</b>	<b>24.1 ± 1.3</b>	<b>54 ± 15</b>	<b>29.4 ± 2.4</b>
Zr	6 ± 4	31 ± 8	0.96 ± 0.56	29 ± 4	21 ± 9	29 ± 13	14 ± 7	7 ± 5	6 ± 2	95 ± 42
Nb	12.4 ± 1.1	16.9 ± 2.7	10.9 ± 1.1	106 ± 9	6 ± 2	14 ± 4	11 ± 3	7 ± 2	12 ± 5	42 ± 9
Mo	173 ± 63	121 ± 22	64 ± 15	306 ± 43	28 ± 2	100 ± 36	66 ± 11	46 ± 17	50 ± 5	468 ± 202
Ru	3.8 ± 0.7	1.4 ± 0.6	3.1 ± 0.2	5.4 ± 0.2	2.8 ± 1.0	4.5 ± 0.3	3.9 ± 0.4	4.2 ± 2.2	11.6 ± 2.1	14.8 ± 3.6
Rh	52 ± 3	94 ± 7	54 ± 7	36 ± 2	61 ± 4	85 ± 29	45 ± 3	58 ± 22	67 ± 10	17 ± 5
Pd	687 ± 16	712 ± 3	676 ± 7	826 ± 110	805 ± 53	648 ± 67	810 ± 74	734 ± 29	743 ± 26	817 ± 18
<b>Ag</b>	<b>3.62 ± 2.51</b>	<b>0.71 ± 0.11</b>	<b>0.54 ± 0.04</b>	<b>0.54 ± 0.06</b>	<b>0.65 ± 0.08</b>	<b>0.61 ± 0.06</b>	<b>1.06 ± 0.29</b>	<b>0.53 ± 0.05</b>	<b>1.83 ± 0.74</b>	<b>1.13 ± 0.28</b>
<b>Cd</b>	<b>0.96 ± 0.12</b>	<b>0.51 ± 0.10</b>	<b>0.85 ± 0.17</b>	<b>2.85 ± 0.57</b>	<b>0.34 ± 0.15</b>	<b>1.57 ± 0.60</b>	<b>0.81 ± 0.10</b>	<b>0.25 ± 0.05</b>	<b>0.74 ± 0.12</b>	<b>2.15 ± 1.25</b>
Sn	138 ± 53	345 ± 78	81 ± 17	73 ± 23	569 ± 332	295 ± 58	147 ± 24	195 ± 69	141 ± 41	669 ± 176
Sb	181 ± 47	153 ± 9	137 ± 34	260 ± 71	124 ± 22	126 ± 45	71 ± 9	112 ± 36	100 ± 17	186 ± 70
Te	8.1 ± 1.1	5.8 ± 0.8	5.7 ± 0.6	39 ± 17	4.0 ± 0.6	12.4 ± 3.2	9.2 ± 0.6	19 ± 10	9.2 ± 0.6	8.0 ± 0.7
<b>I</b>	<b>58 ± 9</b>	<b>37.6 ± 3.2</b>	<b>40.9 ± 2.7</b>	<b>61.3 ± 2.1</b>	<b>39.9 ± 1.9</b>	<b>16.6 ± 1.8</b>	<b>14.3 ± 1.3</b>	<b>31.8 ± 1.9</b>	<b>17.2 ± 2.6</b>	<b>15.1 ± 0.8</b>
Cs	58 ± 18	130 ± 72	31 ± 4	30 ± 7	136 ± 93	45 ± 14	31 ± 5	49 ± 17	35 ± 6	95 ± 38
<b>Ba</b>	<b>1.88 ± 0.68</b>	<b>2.39 ± 1.24</b>	<b>10 ± 2</b>	<b>18 ± 3</b>	<b>1.39 ± 0.44</b>	<b>0.91 ± 0.19</b>	<b>0.86 ± 0.14</b>	<b>1.08 ± 0.16</b>	<b>0.85 ± 0.09</b>	<b>1.69 ± 0.37</b>
<b>La</b>	<b>2.67 ± 0.64</b>	<b>2.92 ± 0.39</b>	<b>2.72 ± 0.60</b>	<b>2.81 ± 0.08</b>	<b>2.38 ± 0.45</b>	<b>4.83 ± 1.90</b>	<b>4.37 ± 1.13</b>	<b>1.85 ± 0.34</b>	<b>6.37 ± 1.02</b>	<b>5.51 ± 2.51</b>
Ce	193 ± 31	186 ± 15	249 ± 15	1640 ± 209	150 ± 19	294 ± 74	205 ± 30	160 ± 60	276 ± 73	309 ± 57
Pr	15.2 ± 0.7	13.4 ± 0.4	16.7 ± 1.3	126 ± 14	8.6 ± 0.8	17.6 ± 3.9	12.2 ± 1.2	12.0 ± 3.4	18.1 ± 5.9	17.5 ± 3.4
Nd	82 ± 5	67 ± 5	82 ± 5	736 ± 74	42 ± 4	86 ± 20	57 ± 6	56 ± 17	89 ± 33	78 ± 15
Sm	13.1 ± 1.5	8.4 ± 0.9	12.0 ± 0.2	127 ± 14	4.7 ± 0.7	11.5 ± 2.8	7.6 ± 0.9	11.0 ± 3.6	13.1 ± 4.7	10.3 ± 1.9
Eu	36 ± 7	29 ± 8	154 ± 34	462 ± 49	17 ± 3	27 ± 6	19 ± 3	25 ± 5	34 ± 13	29 ± 5
Gd	11.2 ± 0.6	8.3 ± 0.9	9.9 ± 0.5	117 ± 12	4.9 ± 0.6	12.8 ± 3.1	8.3 ± 1.1	8.2 ± 1.6	13.4 ± 4.9	10.9 ± 1.6

**Table 2** (continued)

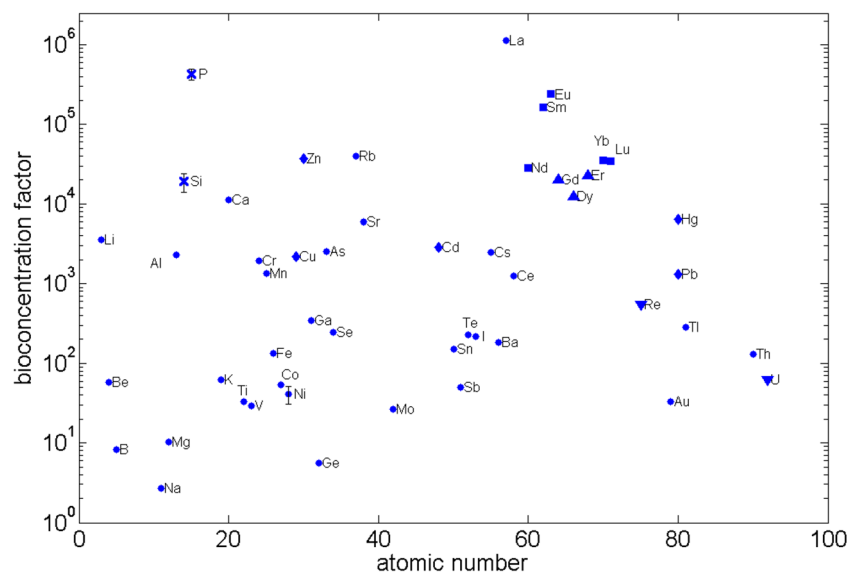
	G (3)	F (3)	M (3)	D+H (3)	A (3)	G2 (10)	G3 (10)	G4 (3)	G5A (7)	G5B (10)
Dy	19.9 ± 0.1	19.9 ± 0.4	19.9 ± 0.3	24.5 ± 3.1	21.0 ± 0.3	17.6 ± 1.9	22.0 ± 2.0	20.4 ± 0.5	19.1 ± 0.4	20.9 ± 0.3
Ho	2.5 ± 0.2	1.7 ± 0.3	2.1 ± 0.2	23 ± 3	1.0 ± 0.2	2.4 ± 0.6	1.4 ± 0.1	1.5 ± 0.2	2.5 ± 0.9	2.0 ± 0.3
Er	26.6 ± 1.5	20.2 ± 2.0	23.0 ± 0.8	123 ± 13	19.3 ± 1.3	23.2 ± 3.3	21.2 ± 1.5	18.4 ± 0.8	23.2 ± 4.7	23.4 ± 1.4
Tm	1.1 ± 0.1	0.61 ± 0.02	0.7 ± 0.1	7.6 ± 0.9	0.24 ± 0.02	0.9 ± 0.3	0.5 ± 0.1	0.4 ± 0.1	0.9 ± 0.4	0.7 ± 0.1
Yb	10.3 ± 1.1	5.2 ± 1.1	7.7 ± 0.0	55 ± 9	2.6 ± 1.1	8.1 ± 2.5	5.8 ± 0.8	5.2 ± 1.1	8.3 ± 3.0	7.0 ± 1.3
Lu	9.6 ± 0.2	5.1 ± 0.4	5.5 ± 0.6	50 ± 6	1.9 ± 0.3	7.5 ± 2.1	5.1 ± 0.8	4.1 ± 0.7	7.6 ± 2.7	5.7 ± 0.7
Hf	165 ± 3	171 ± 6	163 ± 3	206 ± 31	176 ± 7	155 ± 17	190 ± 19	176 ± 8	171 ± 6	179 ± 3
Ta	9.01 ± 0.0	9.0 ± 0.0	6.0 ± 2.5	21.0 ± 2.5	9.0 ± 0.0	9.9 ± 0.9	8.1 ± 0.9	9.0 ± 0.0	9.0 ± 1.8	12.6 ± 1.9
W	200 ± 57	650 ± 119	304 ± 57	125 ± 47	782 ± 371	853 ± 247	273 ± 61	490 ± 188	311 ± 84	731 ± 100
Re	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	1.2 ± 0.2	0.7 ± 0.3	0.7 ± 0.1	0.4 ± 0.1	0.7 ± 0.3	1.0 ± 0.1	0.8 ± 0.1
Os	59 ± 6	26 ± 6	30 ± 3	85 ± 13	26 ± 6	32 ± 4	40 ± 5	97 ± 29	30 ± 6	42 ± 4
Ir	54 ± 6	121 ± 11	58 ± 10	19 ± 3	74 ± 14	82 ± 27	65 ± 8	86 ± 32	70 ± 9	14 ± 1
Pt	54 ± 7	155 ± 19	74 ± 11	47 ± 6	114 ± 16	118 ± 35	79 ± 18	74 ± 42	91 ± 14	31 ± 4
Au	504 ± 230	263 ± 119	321 ± 94	268 ± 71	579 ± 339	267 ± 66	155 ± 36	305 ± 121	146 ± 43	322 ± 68
<b>Hg</b>	<b>0.94 ± 0.27</b>	<b>0.72 ± 0.19</b>	<b>0.68 ± 0.14</b>	<b>0.83 ± 0.10</b>	<b>2.28 ± 1.39</b>	<b>1.33 ± 0.43</b>	<b>0.70 ± 0.13</b>	<b>1.02 ± 0.42</b>	<b>0.58 ± 0.10</b>	<b>0.87 ± 0.13</b>
Tl	16 ± 3	18 ± 3	12 ± 2	40 ± 9	29 ± 17	18 ± 6	6.2 ± 1.0	15 ± 3	2.9 ± 0.5	30 ± 9
<b>Pb</b>	<b>17 ± 4</b>	<b>2.2 ± 0.5</b>	<b>1.7 ± 0.2</b>	<b>7.0 ± 0.2</b>	<b>2.6 ± 0.8</b>	<b>5.1 ± 2.2</b>	<b>1.7 ± 0.2</b>	<b>0.90 ± 0.04</b>	<b>1.4 ± 0.2</b>	<b>4.4 ± 1.8</b>
Th	42 ± 27	47 ± 33	19 ± 6	216 ± 39	512 ± 401	91 ± 58	14 ± 4	57 ± 41	10 ± 4	16 ± 3
U	87 ± 9	387 ± 234	58 ± 12	459 ± 75	17 ± 4	51 ± 14	46 ± 5	59 ± 34	65 ± 20	44 ± 6

maturation stages (except for stage 4). This provides a strong argument in favor of biochemical involvement of these elements in mussel oogenesis. Although no specific function of these elements in the reproductive system of female mussels is known to us at present, there are reports on essentiality of zinc in the development of oocytes and embryos of a frog *Xenopus laevis* (Falchuk and Montorzi 2001), squirrelfish *Holocentrus adscensionis* (Thompson et al. 2012), and mammals (mice) (Kim et al. 2010; Tian et al. 2014). Manganese has been recognized as a vital

element for egg production, growth, and development of chick embryo, and arsenic deficiency has proven to have a deleterious effect on the conception rate and gestation of goats (Mertz 1986).

Mussel male gonads have significantly higher content of phosphorus as compared with female ones. This feature is associated with the increased concentrations of phosphate esters, primarily adenosine triphosphate, which serves as the main energy source for powering the sperm motility (Amelar et al. 1980).

**Fig. 2** Bioconcentration factors for elements in tissues of *M. galloprovincialis*. Concentrations of the elements in the seawater are taken from this study (circles) and the following refs.: Ryabushko et al. (2017) (crosses), Egorov et al. (2018) (diamonds), Colodner et al. (1995) (down-pointing triangles), German et al. (1991) (squares), and Ryabinin et al. (2011) (up-pointing triangles). The 95% uncertainties are shown for three elements (Si, P, Ni) as an example



**Table 3** Comparison between male and female tissues. Elements with significantly higher concentrations ( $p < 0.05$ ) in analogous tissues are presented

	Females	Males
Gills	-	Zn, La, Pb
Foot	-	-
Mantle edge	-	-
Digestive system and heart	-	-
Adductor muscle	K, V, Rb, Ho, Tm	Sb, Pt
Gonads, stage 3	Mg, Si, Ca, Mn, Co, As, Sr, Cd, Pr–Lu	P, Ga
Gonads, stage 4	-	-
Gonads, stage 5A	Mn, Zn, As, Sn	F, P, La
Gonads, stage 5B	F, Mn, Co, Cu, Zn, As, Se, Br, Ru, Rh, Te, Ir	P, K, Cr, Mo, Au
Gonads, pooled stages	Mn, Co, Cu, Zn, As, Br, Nd-Gd, Ho-Lu	P, K, Rb

The lack of significant differences in trace element concentrations in male and female gonads at sexual maturation stage 4 may be related to intensive gonadal tissue growth at this stage, which reduces the concentrations (Lobel and Wright 1982) and smooths out their differences.

There are reports on differences in the accumulation of several trace elements in the somatic and gonadal tissues of mussels (Latouche and Mix 1982; Lobel and Wright 1982). It is interesting to compare the concentrations of a larger number of elements in these tissues in both females and males of mussels. To this end, we apply the two-sample Kolmogorov-Smirnov and Wilcoxon rank sum tests to the aggregate concentrations of each element in gonads and in the other tissues. The concentrations are considered significantly different if the null hypothesis of no difference is rejected in both tests at the significance level 0.05. The results are presented in Table 4. Most of the elements with significantly different concentrations are observed in males (24 elements). Among them, the trace elements except for Ru are predominant in somatic tissue whereas the macroelements (F, Na, P, Ca) except for Si are concentrated in gonadal one.

**Table 4** Elements with significantly higher concentrations in either somatic (s) or gonadal (g) tissue of female and male mussels. The dashes denote the lack of significant differences

	Females	Males	Females	Males	Females	Males		
F	g	g	Ni	s	s	Te	-	s
Na	g	g	Cu	-	s	I	s	s
Si	s	s	Zn	-	s	Cs	g	-
P	-	g	Ge	-	s	Ba	-	s
Ca	-	g	Se	-	s	La	g	-
Ti	-	g	Br	-	s	Eu	-	s
V	s	s	Ru	g	g	Tl	-	s
Mn	-	s	Rh	-	s	Pb	-	s
Co	s	s	Sn	g	-	U	-	s

In females, a considerably smaller number of trace elements (V, Co, Ni, I) are more concentrated in somatic tissue, and the same is true for the macroelements (F and Na) in gonadal tissue of females. On the other hand, unlike males, females are found to contain higher concentrations of such trace elements as Sn, Cs, and La in gonads.

The higher concentration of trace elements in somatic tissue is indicative of their predominant absorption in the digestive system and gills (Pentreath 1973; Regoli 1998) whereas the greater abundance of the macroelements in gonadal tissue apparently reflects the greater content of seawater in gonads (especially at stage 5B) and the need in calcium and phosphorus to support the sperm motility (Alavi et al. 2014; Amelar et al. 1980).

The greater abundance of Ni in somatic tissue observed in Latouche and Mix (1982) is observed in our results, too, but the higher concentrations of such trace elements as Zn, Mn, and Cu in somatic tissue are reliably confirmed only for male specimens, and there is no difference of cadmium concentrations in gonadal and somatic tissues of both males and females, according to our data.

A much smaller number of elements with significantly different concentrations in either somatic or gonadal tissue of mussel females indicate faster element translocation and possibly more intense metabolism than in males.

### Effects of the sexual maturation stages on the trace element accumulation in gonads

As pointed out above, the gonads of the sampled mussels were categorized according to the six-stage scale of ripening. Because gonads grow with unequal rates and may contain unequal amounts of elements at different maturation stages, it is essential to study the concentrations of elements in gonads over the reproductive cycle. It is also important to measure the trace element composition of gonads at two substages of stage 5 (before and immediately after the spawning), which are referred to in this work as stages 5A and 5B, because elements

can be released with the sex products and because the gonad mass decreases after the spawning.

### Significantly different concentrations of elements in gonads of different sexual maturation: nonparametric testing

To estimate the differences in the trace element accumulation in the female and male gonads, we use the concentrations of each element as the observations and the sexual maturation stages as the groups and check the overall distinguishability among these groups using the Kruskal-Wallis and Welch ANOVA tests. Once the distinguishability has been detected, the Dunn or Games-Howell tests, respectively, are applied as a post hoc procedure of multiple pairwise comparisons using rank sums (Dunn 1964).

The results of using this test are summarized in Table 5. In total, 22 elements for females and 17 elements for males have significant differences in their gonadal concentrations at least in one pair of the ripening stages. The mean concentrations of these elements normalized to their mean over all stages are shown in Figs. 3 and 4.

For female gonads, the following patterns of the element concentrations can be identified: (a) concentration increase at stage 5B as compared with one or both “intermediate” stages (4, 5A), which appears in Fig. 3a as a minimum at these stages although the minimum is statistically confirmed only for Li, Na, and Mg; (b) concentration increase at stage 5B as compared with stage 3 and/or 4, which is seen in Fig. 3b as the steady accumulation over the four stages; (c) apparent concentration increase at “intermediate” stages 4 and 5A (significant for Rh, I, and Pt in Fig. 3c); and (d) significant concentration decrease at stages 4 and 5A (As, Br, Cd). There are 18 elements (82 %) with significantly higher concentrations at stage 5B and only 4 elements (18 %) with higher concentrations at other stages.

In the male gonads, the tendency of the concentration increase at stage 5B is clearly seen, too (Fig. 4a). The other patterns include the accumulation at stage 3 and/or 5A typically accompanied by the concentration decrease at stages 4 or 5B (Fig. 4b), which are likely related to the biomass dilution at stage 4 (F, Cd, La, Pb) and release with sperm at stage 5B (Rh, Ir, Pt). A special case is iodine, which demonstrates significantly higher concentrations at stage 4. The number of the elements with the significantly higher concentration at stage 5B in the male gonads is 8 (47%) as compared with 9 elements (53%) more concentrated at other stages.

Generally, the tendency of the element concentration increase in gonads after spawning is in agreement with the inverse relationship of the body weight and element concentration (Boyden 1974; Cossa et al. 1980; Phillips 1976; Simpson 1979) as mollusks lose up to 40% of their wet weight during the spawning (Cossa 1989). It also suggests that a large fraction of trace elements is not accumulated in mussel gametes.

The Kruskal-Wallis and Welch ANOVA tests are reliable enough for detecting differences among medians of samples, but they do not provide information about the distributions, their quantitative characteristics, and outliers from these distributions. This encourages us to introduce an additional method of statistical processing of the results, and such an approach can be analysis of distributions of normalized concentration increments for any pair of the sexual maturation stages as given below.

### Significantly different normalized concentration increments in gonads of different sexual maturation: analysis of distributions

Consider the gonadal element concentrations normalized to the mean for all sexual maturation stages available. The random deviations from a mean increment of these concentrations in a pair of maturation stages form a distribution of increments. From the Lilliefors and Shapiro-Wilk tests (implemented as the Matlab Statistics Toolbox functions *lillietest* and *swtest*), these distributions are not normal, and they fit well into Student’s *t* location-scale statistics (Fig. 7) with the probability distribution function:

$$p(x) = \frac{\Gamma(\nu/2 + 1/2)}{\sigma\sqrt{\nu\pi}\Gamma(\nu/2)} \left[ 1 + \frac{1}{\nu} \left( \frac{x-\mu}{\sigma} \right)^2 \right]^{-\frac{\nu+1}{2}} \quad (2)$$

where  $\Gamma$  is the gamma function,  $\mu$  is the location parameter (median),  $\sigma$  is the scale parameter proportional to the square root of variance,  $\nu$  is the shape parameter (number of degrees of freedom), and  $x$  is the mean-normalized concentration increment between two stages:

$$x_{E,ij} = \left( \bar{C}_{E,\text{stage } i} - \bar{C}_{E,\text{stage } j} \right) / \langle \bar{C}_E \rangle \quad (3)$$

in which  $\bar{C}_{E,\text{stage } i}$  is the mean concentration of element  $E$  in the gonads at sexual maturation stage  $i$  and  $\langle \bar{C}_E \rangle$  is the mean for all stages.

It is accepted to detect outliers ( $x_{out}$ ) in non-normal distributions of a quantity  $x$  using the rule of three scaled median absolute deviations (MAD) around the median (Leys et al. 2013):

$$\left| x_{out} - \underline{\underline{\{x_i\}}} \right| / \text{MAD} > 3 \quad (4)$$

where

$$\text{MAD} = \frac{\text{erfc}^{-1}(3/2)}{\sqrt{2}} \left\{ \underline{\underline{\{x_i - \underline{\underline{\{x_j\}}}\}}} \right\} \quad (5)$$

and  $\underline{\underline{\{\cdot\}}}$  denotes the median of a data set.



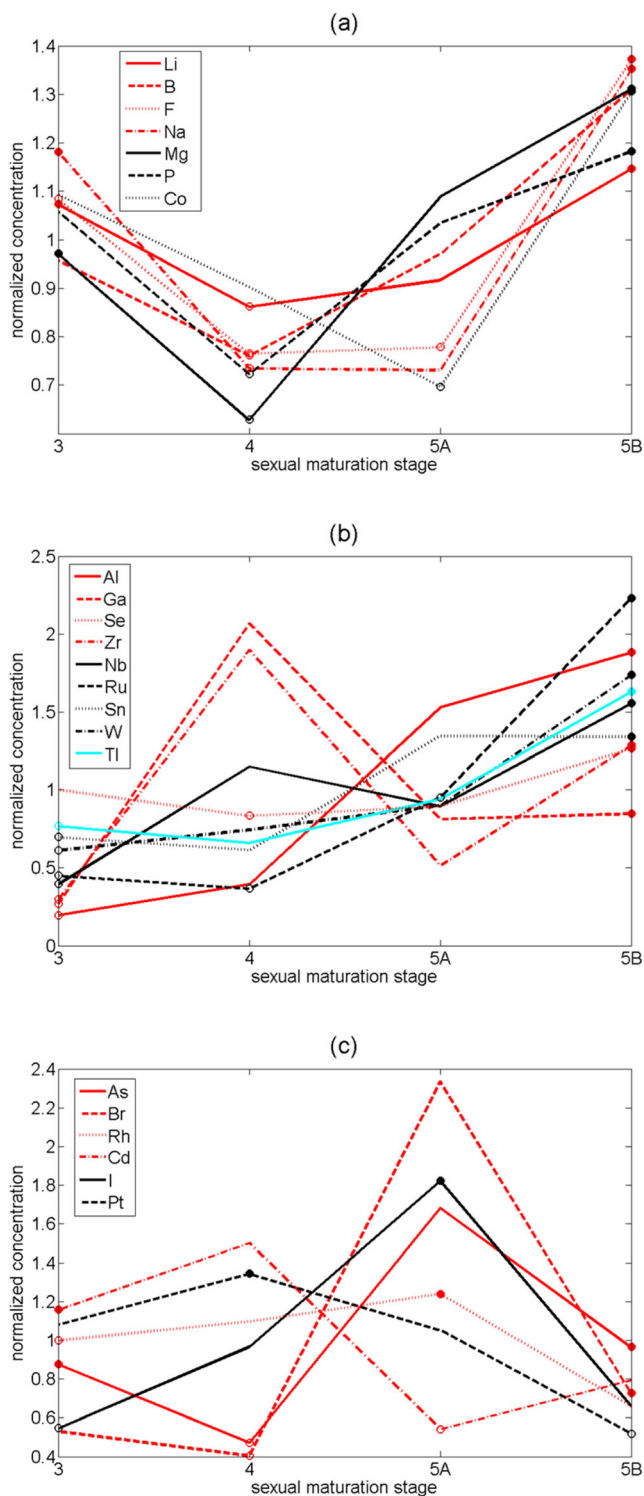
**Table 5** Testing significance of differences of the element concentrations and concentration increments in female and male gonads at different maturation stages (see the text). The stages with significant differences are shown as (lower)-(higher) value stages, and the values of each stage in parentheses differ significantly from those of its hyphen-separated counterpart

	Concentration: the Kruskal-Wallis and Dunn’s tests or Welch ANOVA followed by the Games-Howell test (*)		Concentration increment: distribution outliers	
	Females	Males	Females	Males
Li	4-(3,5B)*	-	-	-
B	4-5B	-*	-	-
F	(3,4,5A)-5B*	4-(3,5A)*	-	-
Na	4-(3,5B)*	4-(5A,5B)*	-	-
Mg	4-(3,5B)*	4-(5A,5B)*	-	-
Al	3-5B	-	3-(5A,5B)	-
Si	-	3-5A*	-	-
P	4-5B*	(3,4,5A)-5B*	-	-
Ca	-	-	(3,5B)-5A	(2,3,4,5B)-5A
Ti	-	-	-	(3,4,5A)-5B
Co	5A-5B*	-	-	-
Ni	-	-	5B-3	-
Cu	-	-	-	5A-2
Zn	-*	4-5B*	-	-
Ga	3-5B	2-5B	3-4	(2,3,4,5A)-5B
As	4-(3,5B)*	-	5B-5A	-
Se	4-5B*	-	-	-
Br	4-5B*	-	(3,4,5B)-5A	-
Zr	3-5B*	5A-5B	(3,4)-5B	(2,3,4,5A)-5B
Nb	3-5B	-	3-5B	(2,3,4,5A)-5B
Mo	-	-	-	(2,3,4,5A)-5B
Ru	(3,4)-5B*	-*	(3,5A)-5B	3-5A
Rh	5B-3*	5B-(3,5A)*	-	-
Cd	5A-3*	4-(3,5A)*	-	4-5B
Sn	3-5B	(3,5A)-5B	-	(3,4,5A)-5B
Te	-	-	(3,5A,5B)-4	(3,5B)-4
I	3-5A	(2,3,5A,5B)-4*	(3,5B)-5A	(3,5B)-4
La	-*	4-5A	-	-
Sm	-	-	(3,5A,5B)-4	-
Eu	-	-	3-4	-
Ta	-	-	(3,5A,5B)-4	-
W	(3,4)-5B*	3-5B*	(3,5A)-5B	-
Re	-	-	(3,5A,5B)-4	-
Os	-	-	(3,5A,5B)-4	(2,3,5A,5B)-4
Ir	-	5B-(3,5A)*	-	5B-4
Pt	5B-3*	5B-5A*	5B-3	-
Au	-	-	5B-5A	-
Hg	-	-	5B-5A	-
Tl	3-5B	-*	3-5B	5A-(2,5B)
Pb	-	4-3*	-	5A-2
Th	-	-	(3,4,5B)-5A	(3,5A,5B)-(2,4)

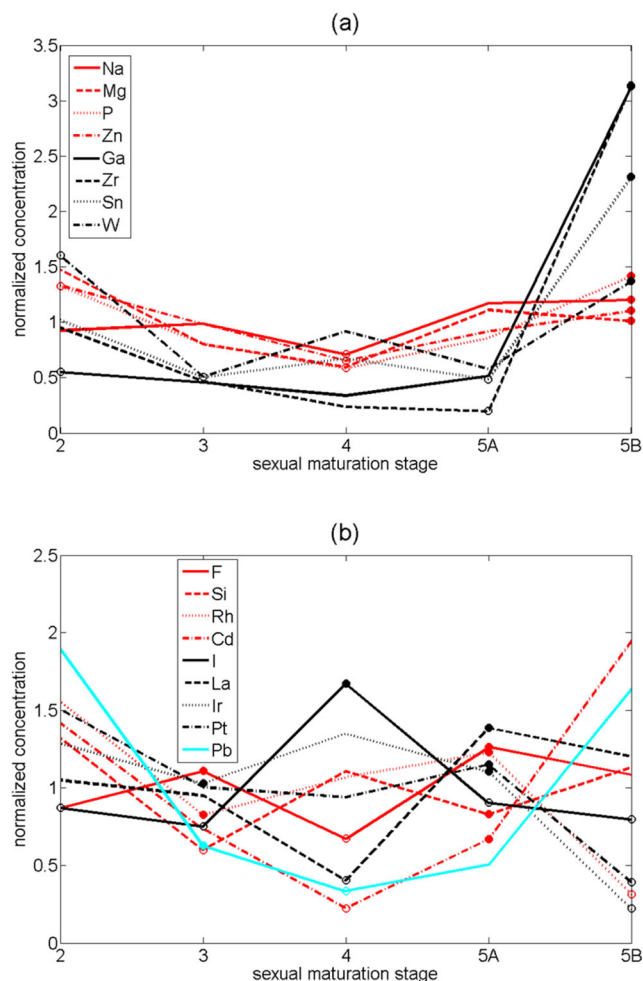
After fitting the increments of normalized concentrations between two stages to distribution (2) using the Matlab Statistics Toolbox app *dftool*, one can thus find outliers from (4) and (5). In Fig. 7, the borderlines for outliers are demarcated with vertical-

dashed lines. To save space, we do not present the increment distributions for individual pairs of sexual maturation stages.

The distribution outliers are the concentration increments for elements that do not lie within the main peak and



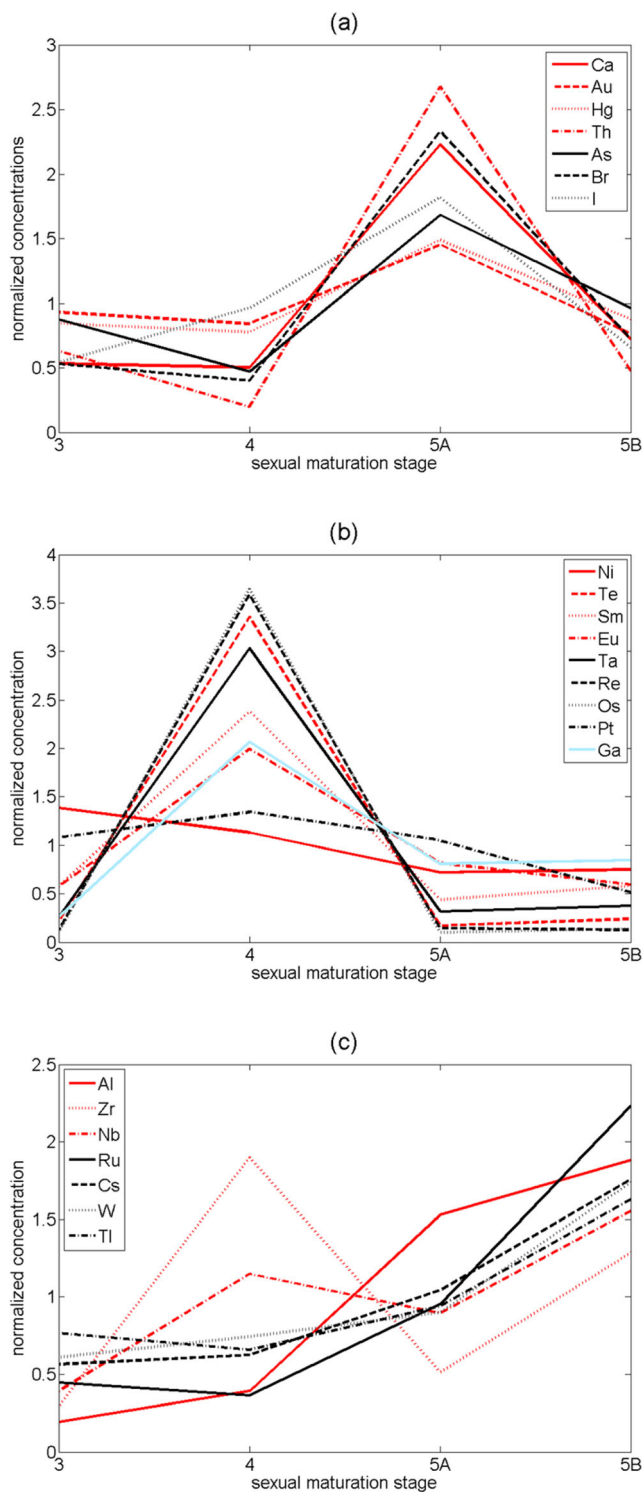
**Fig. 3** Mean normalized concentrations of elements in gonads of female mussels with significant differences in at least one pair of the sexual maturation stages. Open symbols, significantly lower concentrations; filled symbols of the same kind, their significantly higher counterparts. (a) Significantly lower concentrations at stages 4 and/or 5A and significantly higher ones at stage 5B, (b) significantly lower concentrations at stages 3 and/or 4 and significantly higher ones at stage 5B, (c) apparently higher concentrations at stages 4 and 5A (significant for Rh, I, Pt), and significantly lower concentrations at stages 4 and 5A (As, Br, Cd)



**Fig. 4** Mean normalized concentrations of elements in gonads of male mussels with significant differences in at least one pair of the sexual maturation stages. The symbols have the same meaning as in Fig. 3. (a) Significantly higher concentrations at stage 5B. (b) Significantly higher concentrations at stage 3 (F, Rh, Ir, Pb) and/or stage 5A (the other elements except for I and Pb) and significantly higher concentration at stage 3 (I)

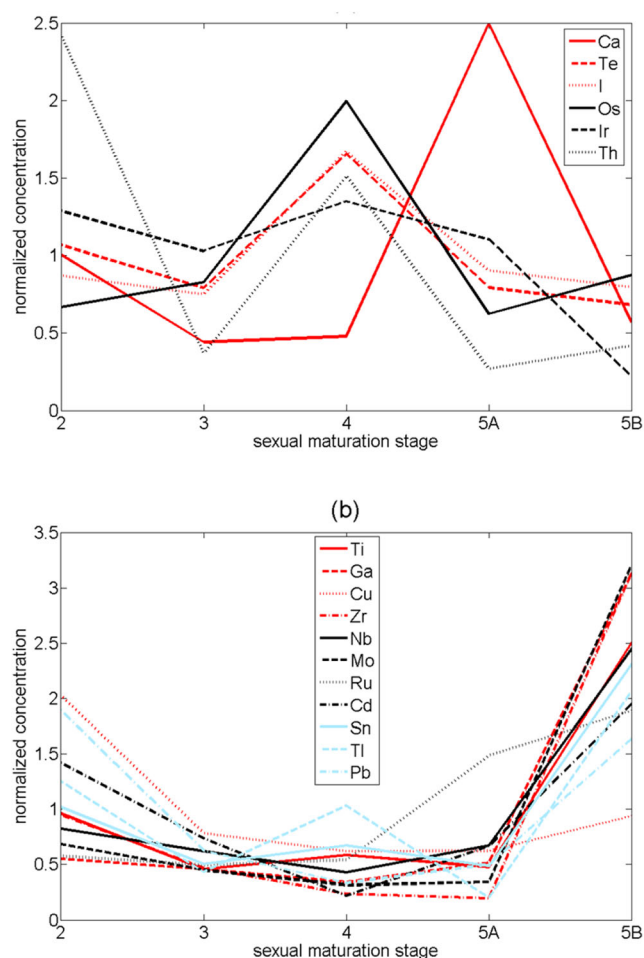
significantly differ from the median for some nonrandom reasons. The elements corresponding to outliers in the increment distributions in female and male gonads are shown in Table 5, and they are referred to as the elements with significantly different concentration increments. The normalized mean concentrations of the elements with significantly different concentration increments are shown in Figs. 5 and 6.

It is seen that the number of the “deviant” increment elements is the same as those with significantly different concentrations (22 for females and 17 for males). However, only about 50% elements from the significantly different concentrations list are found in the list of significantly different increments of normalized concentrations (11 for females and 7 for males). This mismatch can be explained by heavy-tailed element concentration distributions which, although being significantly different in their individual medians according



**Fig. 5** Mean normalized element concentrations in gonads of female mussels with significantly different increments in at least one pair of the sexual maturation stages. (a) Significantly higher increments at stage 5A, (b) significantly higher increments at stages 3 (Ni, Pt) and 4, and (c) significantly higher increments at stage 5B

to the non-parametric ANOVA tests, result in near-zero increments of the mean normalized concentrations between two stages.



**Fig. 6** Mean normalized element concentrations in gonads of male mussels with significantly different increments in at least one pair of the sexual maturation stages. (a) Significantly higher increments at stages 4 and 5A (Ca) and (b) significantly higher increments at stages 2 (Cu, Pb) and 5B

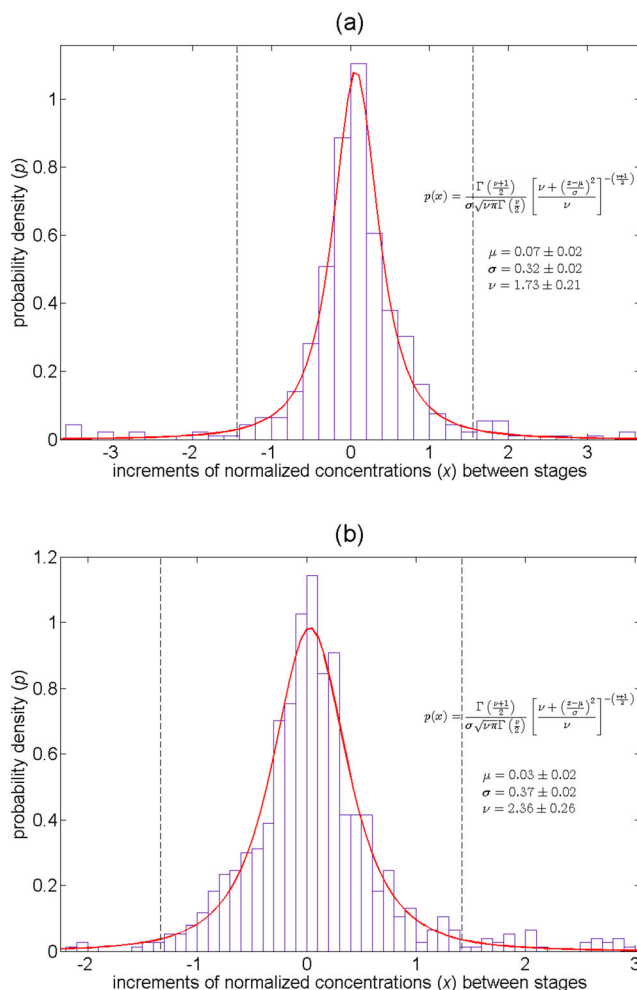
On the other hand, the elements with significantly different increments that are not found among the elements with significantly different concentrations can be related to essentially different values of outliers in the heavy-tailed concentration distribution, which are not very important in the non-parametric tests, but strongly affect the means of two populations. Many of these elements demonstrate distinct maxima at stages 4 and 5B in Figs. 5a, b, and 6a. It is likely that these maxima result from occasional capture of rare rock or seabed microparticles that are not evenly dispersed in the water layer in which mussels reside. A similar finding of rare elements sourced from suspended particles in bivalves was reported in Akagi and Edanami (2017). Female mussels at stages 4 and 5A can be more active in filtering out such microparticles, or retain them for a longer period of time, or capture finer particles so that a much larger number of elements in female gonads than in male ones have the increased mean concentrations at these stages (14 elements in female against 7 ones in male gonads). However, it should be again emphasized that

the differences in the concentrations of most of these elements between stages are not statistically significant and are mainly due to the heavy-tailedness of the concentration distributions.

Six of nine elements with significantly higher concentration increments at stage 5B in female gonads (Fig. 5c) have also significantly higher concentrations at this stage as compared with stage 3 (Fig. 3b). On the contrary, only three of eight elements with significantly higher concentration increments in male gonads (Fig. 6b) are among the elements with significantly higher concentrations at stage 5B (Fig. 4a). This discrepancy can be explained with the existence of positive outliers in the male gonad concentrations as male gonads at this stage have occasionally smaller acinar lacunas than those in female gonads. These lacunas with high surface area previously filled with germ cells are filled with seawater after the spawning. The trace elements are adsorbed on their walls and in the ducts, and thus, the male gonads can occasionally contain higher concentrations of the elements carried by seawater.

The distributions of aggregate concentration increments in all pairs of the maturation stages of female and male gonads are shown in Fig. 7 together with the corresponding *t* location-scale fits. It is seen that the distribution medians are slightly above zero, indicating an overall accumulation of elements throughout the range of the sexual maturation stages under study, in agreement with the abovementioned tendency. The mean for female gonads (0.07) is approximately twice as high as that for male ones (0.03), most likely because during the first stages (1 and 2) of the reproductive cycle, the overall trace element depletion occurs in gonads, but female mussels at both these stages and male mussels at stage 1 were not found in the sampling period. Thus, the distribution mean for all stages of the reproductive cycle is expected to be zero within the fit accuracy in both female and male gonads, and the gonadal concentrations of most elements with constant background levels, integrated over the reproductive cycle, are conservative. This, of course, does not disprove the possible accumulation of elements in time because the sampling was performed in only one season, when the element concentrations in the environment are approximately constant and relatively low.

To support the above speculations about the distribution medians, it is possible to estimate the overall tendency of the mean concentrations of elements in gonads to increase, decrease, or remain unchanged between two stages. The sets of the mean concentrations at all pairs of the ripening stages are taken as two paired populations, and the one-sided Wilcoxon signed rank test is applied to them. In female gonads, all significant differences are positive and there is a steady trend of the concentration increase starting from stage 3 (Table 6). In male gonads, the concentrations drop after stage 2 and start increasing from stage 3. Thus, from stage 3 on in gonads of both sexes, the element concentrations demonstrate the overall increase whereas at stage 1 (missed for mussels of both sexes)



**Fig. 7** The probability density (PD) histograms of normalized concentration increments in all pairs of the maturation stages of (a) female and (b) male mussel gonads. The dashed vertical lines demarcate the intervals of three scaled median absolute deviations. The PD function fit line of the *t* location-scale distribution and the fit parameters are shown in the panels

and stage 2 (missed for females), they apparently tend to decrease.

The ceiling values of the fitted shape parameter  $\nu$  in the Student *t* location-scale fits in Fig. 7 are 2 for female gonads and 3 for male ones. These two values are in a good agreement with the meaning of the number of degrees of freedom as the number of variables (i.e., the number of sexual maturation stages under study) minus one. The *t* location-scale

**Table 6** Pairs of sexual maturation stages with significant differences between paired populations of element concentrations, according to the Wilcoxon signed rank test

	Positive differences	Negative differences
Female gonads	-	3-4, 3-5A, 3-5B, 5A-5B
Male gonads	2-3, 2-4, 2-5A	3-5A, 3-5B, 4-5A, 4-5B, 5A-5B



distribution represents the distribution of random deviations in systems with low degrees of freedom. This suggests that the normalized concentration increments that fit into this distribution follow the common periodic accumulation pattern over the reproductive cycle. The suite of elements that exhibit this pattern incorporates, in particular, some elements with significant concentration differences between maturation stages, including macroelements (except for Ca) and some trace elements from Table 5, such as Li, B, Co, Zn, Se, and Rh.

It should be borne in mind that the distribution analysis presented in this section is to some extent compromised by the use of means at individual stages because the use of means does not make much sense if the concentration distribution within a maturation stage considerably differs from Gaussian. Nonetheless, this analysis is a powerful tool for detecting general trends over the reproductive cycle and is a good supplement to the nonparametric statistics when analyzing the effects and origins of outliers.

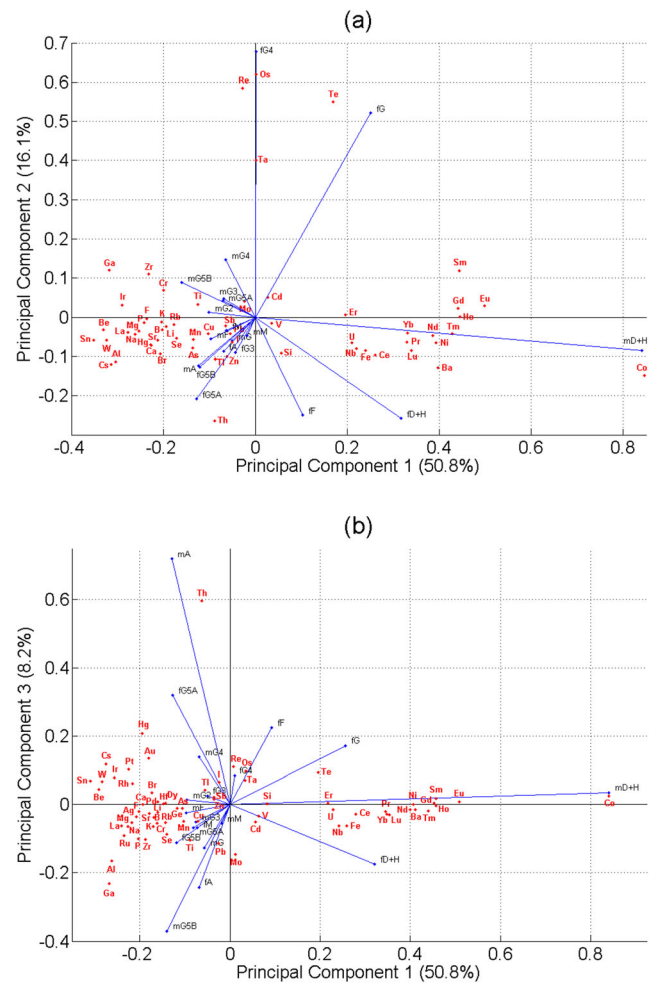
### Multivariate analysis of element concentrations

#### Principal component analysis

To study the contribution of each soft tissue in the total element composition and to explore some basic regularities of element accumulation in male and female mussel tissues, principal component analysis (PCA) of element concentrations normalized by their tissue-averaged values was performed.

The PCA with tissues as variables and elements as observations yields the principal component (PC) variances and the percentages of the total variance explained by each PC, first ten of which are shown in Table S3 (Supplementary Material). Variances of the first three PC explain 75% of the total variance, and thus, use of only three dimensions is sufficient to account for most of concentration variations in different tissues of male and female mussels.

In Fig. 8, a 3D biplot with observations as points and variables as vectors is shown in two projections. One can see that the vectors of female foot, gills, and digestive system + heart of males and females are aligned with the positive direction of the first PC (PC1) axis. A particular great contribution to PC1 is made by the vector of male digestive system + heart, along which observations for such elements as Fe, Co, Ni, V, Nb, and Ba, and lanthanides (except for La and Dy) are dispersed. Mussel gills are believed to non-specifically absorb trace elements through passive diffusion (Carpenè and George 1981; Elliott et al. 1985; van Haren et al. 1990; Langston and Bryan 1984). The element uptake via gills is typically lower than the accumulation in digestive gland and usually exceeds that in the remaining tissues (Abderrahmani et al. 2020; Odzak et al. 2000; Pentreath 1973; Regoli 1998) although this pattern is not universal and depends on the element identity (Raspor



**Fig. 8** Principal component analysis, tissue-mean-normalized concentrations of elements as observations and tissues as variables. Tissue notation (black), f/m denotes female/male and the tail abbreviations denote the tissue names as in Tables 1 and 2. Percentage of the variance explained is given in parentheses (in the axes)

et al. 1999; Yabanlı et al. 2015), tissue, and sex (Tables 1 and 2).

In the negative direction of PC1 axis, one can see vectors of male and female gonads at different maturation stages, adductor muscle, and mantle edge. Many elements, including all macroelements, are bunched in the negative half-space of PC1. This is understandable since the digestive system and gills in the mussel organism have a smaller relative weight than the other tissues, which accumulate a greater variety of elements. Thus, it is reasonable to assume that the positive direction of PC1 is mainly related to the uptake of the elements in the digestive tract and the negative direction of PC1 corresponds to their redistribution in the other tissues.

A strong contribution to the positive direction of the second PC (PC2) is made by the variables of female gills and gonads at stage 4, and the rare elements Re, Os, Ta, and Te are localized in the vicinity of these vectors. It is likely that the filtration through the gills of females is increased at stage 4, when

the female gonads grow fast due to the intensive development of eggs. During the active filter-feeding at this stage, female mussels may capture suspended rock microparticles and accumulate greater amounts of rare elements, such as Zr, Nb, Te, Hf, lanthanides, Re, Os, Ir, and Pt, in their gonads. However, as shown above, this uptake is accidental and contingent on availability of rare-element-enriched microparticles captured together with plankton.

Furthermore, the variables of the male gonads at all stages contribute to the positive direction of PC2, and the vectors of all the other female and male tissues are oriented in the negative direction of PC2. An unexpected result of the nearly opposite orientation of the vectors of the female and male gill variables is a token of the sex-related differences in the physiological function of gills in male and female mussels. As mentioned, these features may be due to the sex-specific trace element utilization in this organ.

There is sex specificity in the processes associated with the third PC (PC3), too. It is seen that the variables of only three analogous tissues (gonads at stages 4 and 5B and the mantle edge) in mussels of both sexes point in the same direction with respect to PC3. This suggests that the processes related to PC3 and common for both sexes are the biomass dilution at stage 4 and the gamete release, but it is difficult to distinguish processes responsible for the sex-specific differences in PC3.

### Clustering of element concentrations in mussel tissues and estimation of optimal clustering

The non-normalized concentrations of trace elements (in ppm) in pooled soft tissues of female and male mussels have been subjected to agglomerative hierarchical cluster analysis using Ward's method. The resulting dendrogram shown in Fig. 9 is unilateral, and it reflects, on the whole, the abundances of elements in the marine environment.

The elements in this dendrogram are almost perfectly sorted in the order of the concentration increase, and this series allows easily spotting element concentration anomalies, e.g., unusually high levels of the rare-earth metal lanthanum, which is situated between copper and lead far away from other lanthanides, and strong accumulation of titanium, which proves to be nearly as abundant in the tissues as iron. The possible reasons for these anomalies are discussed above.

The nonparametric statistical energy method both in the Aslan-Zech and Székely-Rizzo interpretations (see the “Materials and methods” section) demonstrates statistical distinguishability of, at most, five non-overlapping clusters of elements obtained using the  $k$ -means clustering procedure in the male and female mussel tissues at the 95% significance level.

On the other hand, the  $k$ -means algorithm can be applied to find also an optimal number of clusters of the element concentrations in the mussel tissues using, as a criterion, a sum of

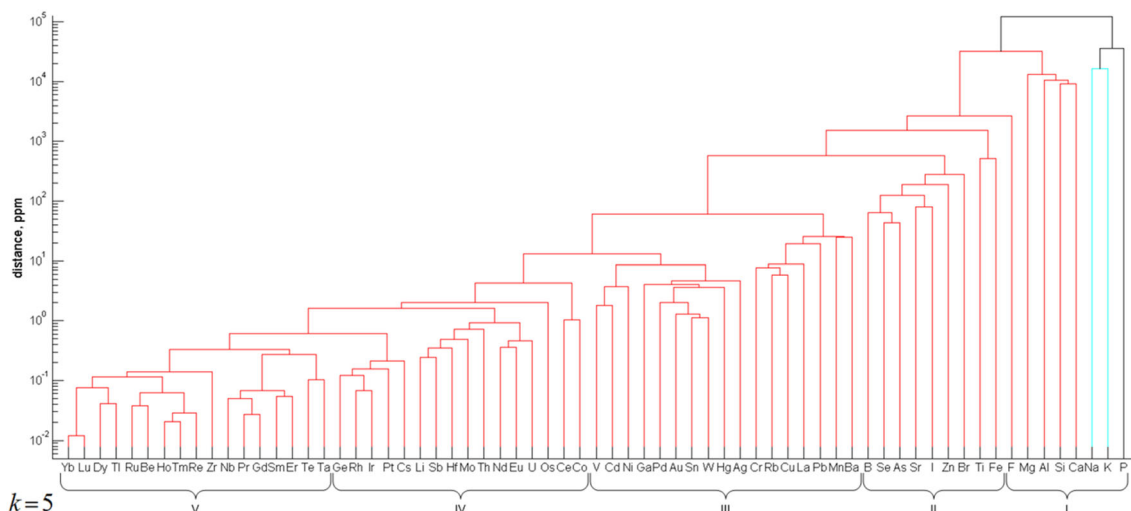
within-cluster sums of logarithms of distances to centroids. The consecutive performing of  $k$ -means clustering with  $k$  ranging from 1 to 11 yields the  $k$ -dependent sums of distances to centroids shown in Fig. 10. These sums can be fitted with two exponential functions, and the best number  $n$  of the successive points in the first fit function can be found from the minimum of the sum of the two mean squared residuals (inset in Fig. 10). Then, the  $k$  value of the intersection point coordinate rounded to the nearest integer can be considered the optimum number of mutually exclusive clusters. In our case, this number is five. In Fig. 9(a), it is shown how the elements are distributed in these five clusters.

The group I of major elements contains the most abundant alkaline and alkaline earth metals, aluminum, fluorine, and some essential nonmetals (phosphorus, silicon). This group is made up of the essential macroelements and the elements (either essential or not) which have a high accumulation potential and high levels in the mussel tissues. It is worthwhile to note that the concentration of fluorine in this work has been determined semi-quantitatively, and its actual cluster allocation is questionable.

Group II consists of essential and possibly essential trace elements and a couple of elements (Ti and Sr) that are relatively abundant in seawater. It is noteworthy that titanium is in the same binary node as iron, its concentration is nearly the same as that of iron, and both elements approach group I of macroelements. This suggests the considerable level of seawater contamination with titanium.

Group III contains some essential elements, e.g., Cu (Taylor and Maher 2016), a number of possibly essential trace elements, and non-essential trace elements, many of which are toxic heavy metals (Pb, Cd, Hg, Ag). This group is characterized by moderate concentrations of elements. Among the non-essential heavy metals, lead has the highest mean concentration in the mussel tissues, which is a consequence of the wide technical use of this metal, e.g., in rechargeable batteries. Surprisingly, this group contains also lanthanum in its top-node cluster although all other lanthanides are in groups 4 and 5 of elements with much smaller concentrations. The bioaccumulated concentrations of lanthanum are about the same as, and in some tissues even higher than, those of lead.

As mentioned, a possible reason for the increased lanthanum concentrations is the landfill erosion and fertilizer leaching which serve as a local source of lanthanum in the seawater. Together with gold and mercury, La demonstrates higher concentrations than the medians of the measurements given in (Eisler 2010). Conversely, the concentrations of such heavy metals as vanadium, nickel, and copper are lower than the corresponding median values, and the concentrations of the other elements in this group are comparable with the medians from the mentioned compendium (Eisler 2010). An explanation to this observation can be found in the fact that sediments that make up the seabed in the coastal area of



**Fig. 9** Dendrogram of the aggregative hierarchical clustering of element concentrations (in ppm) in pooled soft tissues of mussels. Ward’s method is used to pair clusters. The results of the optimum  $k$ -means clustering ( $k = 5$ ) are additionally shown below the panel

Crimea are depleted in heavy metals and some of these metals (e.g., Cu, Ni, and V) can be efficiently removed from the water column by adsorption on particulate organic matter. On the contrary, freshwater runoff is likely the persistent source of presumably natural mercury and gold and presumably anthropogenic lanthanum in the seawater.

Lastly, groups IV and V of the dendrogram in Fig. 9 include non-essential rare (ultratrace) elements, in particular, lanthanides and precious metals, which are in very low amounts (below  $\sim 1$  ppm dw) in the tissues and do not have any known physiological function.

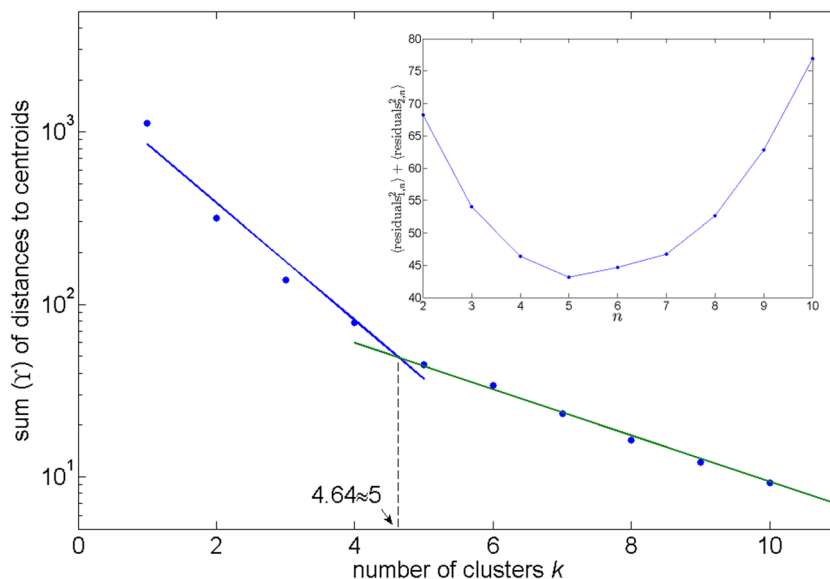
It is worthwhile to note that essential, at least for mammals, are almost all elements from groups I and II (Fig. 9) and several elements from group III (Mn, Cu, and possibly Cr), which are in the cluster of the most abundant ones in this group. This observation supports the hypothesis that the trace

element requirements of living organisms must reflect to some extent the availability of these elements in the environment (Valkovic 1990).

### Conclusions

In this study, concentrations of an unprecedentedly large number (67) of elements in six types of soft tissues of females and males of the Mediterranean mussel *M. galloprovincialis* living in the coastal waters off Sevastopol (southwestern Crimea, Black Sea) have been quantified using ICP-MS. The data obtained have allowed calculating the bioconcentration factors and have been aggregately used, e.g., in the principal component analysis and cluster analysis.

**Fig. 10** Finding the optimum number of clusters from the point of intersection of two best exponential fits of summed distances to cluster centroids



Statistical differences in the element composition of analogous tissues of female and male mussels have been detected. For example, gills of males have been found to contain significantly higher concentrations of Zn, Pb, and La, which may be due to sex-specific metal transportation in this organ, whereas gonads of males have higher concentrations of phosphorus and gonads of females are richer in such elements as Zn, Mn, and As, indicating probable essentiality of these elements in gametogenesis.

Significantly higher concentrations of many trace elements have been detected in somatic tissue of male mussels as compared with their gonadal tissue, and male gonads contained higher concentrations of macroelements. On the contrary, much fewer elements have been found to have significant differences in somatic and gonadal tissues of female mussels, thus supporting an idea that element translocation processes (and likely, metabolism) are more intense in the female mollusks.

For the first time, particular attention has been paid to peculiarities of the element accumulation in mussel gonads at different stages of sexual maturation. Several accumulation patterns have been detected using nonparametric tests. Among these patterns, the most pronounced and general one for both female and male gonads is the significant concentration increase at stage 5B after the spawning, which has been attributed to the well-known effect of the mussel weight reduction. An original statistical approach based on the distribution analysis and applied to mean normalized concentration increments between stages has confirmed this general trend, but also revealed heavy-tailed concentration distributions with outliers that compromise appropriateness of using means in this analysis. Furthermore, the results of using both this approach and the signed rank test have shown that concentrations of most elements in mussel gonads likely circulate over the reproductive cycle, tending to decrease over the first two sexual maturation stages and to increase at the subsequent stages.

The lack of the concentration differences in male and female gonads due to the intense gonadal growth has been observed at stage 4 rather than at the general minimum concentration stage 3. Due to this fact and because the concentrations generally start increasing after stage 3, i.e., long before the gamete release, the element circulation casts doubts on the passive biomass dilution as the only reason for the trace element concentration decrease in gonads. It is likely that active sequestration of trace elements in the first half of the reproductive cycle also contributes to this decrease.

The results obtained suggest that when monitoring water quality using mussels' gonads, a considerable number of animals (no fewer than about a dozen) should be collected to ensure the adequate statistics and the effect of sexual maturation should be taken into account. The sexual maturation is expected to affect the element concentrations not only in

gonads, but also in other mussel tissues of both males and females. This problem has not been investigated to date and is worth studying in the future.

The principal component analysis of element concentrations normalized to their mean values has demonstrated that the main factors affecting the element distribution, in the order of their comparative importance, are the uptake via the digestive system and gills and the sexual differentiation. Accordingly, the most important identified factors determining the decrease in concentrations of tissue elements are their redistribution over mussel's body, dilution with biomass at the stage of intense gonadal growth (and likely, active sequestration at early gonadal ripening stages), and release with gametes.

The hierarchical cluster analysis performed by Ward's method using non-normalized concentrations of elements in pooled mussel tissues has proven to be useful in spotting trace elements with anomalously high concentrations, such as titanium and lanthanum. The resulting dendrogram clearly shows that mussels preferably accumulate essential, competing, and abundant elements, in line with the results of (Richir and Gobert 2014). Furthermore, the *k*-means clustering applied has allowed detecting an optimum of five non-overlapping clusters of elements in the tissues using the nonparametric statistical energy method and the criterion of bending in the logarithm of summed within-cluster distances plotted against *k*. The five clusters have been related to (a) a group of most abundant elements, including essential macroelements; (b) a group of essential and abundant trace elements; (c) a group with non-essential and a few essential and possibly essential trace elements; and (d) two groups of non-essential ultratrace elements. This grouping in the future can lay a foundation for the more rigorous, non-arbitrary division of elements into macroelements, trace, and ultratrace elements in biological tissues.

This work allows deciding which tissues from mussels of which sex should be used to increase the analysis sensitivity when quantifying certain trace elements in the mussel biomonitoring. The highest concentrations of many heavy metals, including Co, Ni, Cu, and Cd, are found in digestive system of males. Such elements as Zn and Pb are most concentrated in male gills. The heavy metals Cr and Sn are at their highest levels in male gonads after the spawning (stage 5B), whereas Mn is most accumulated at the same sexual maturation stage in female gonads. Arsenic has peak concentrations in female gonads at the spawning stage, and mercury is approximately equally distributed over all tissues. It is worthwhile to note that these regularities are not absolute and seem valid for relatively clean water areas, such as the south coastal area of Crimea. In areas with higher loadings of trace elements, the order of their distribution in mussel tissues may be different, and the highest concentrations are likely to occur in male and female gills and digestive system.



**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11356-020-12024-z>.

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**Authors' contributions** SVK wrote the initial draft, edited the final version of the manuscript, and contributed to the ICP-MS and data analysis. NVK carried out the field work, sex and sexual maturation stage discrimination, and preparation of tissue samples. NIB prepared analytical samples and contributed to the ICP-MS analysis. VIR conceived the idea and developed the research design. LLK contributed to the data analysis.

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**Data Availability** The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## Compliance with ethical standards

**Ethics approval** Not applicable

**Consent to participate** Not applicable

**Consent for publication** Not applicable

**Competing interests** The authors declare that they have no competing interests.

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