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Title: Biochemical composition of freshwater mussels in Malaysia: a neglected nutrient source for rural communities

Abbreviated title: Heavy metals and nutritional value of Malaysian freshwater mussels

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Abstract

Nutrient deficiency is still prevalent in Southeast Asia. Freshwater mussels (Unionida) are widespread and consumed by low-income, rural and indigenous communities, but their biochemistry is poorly known. We assessed concentrations of nutrients and harmful heavy metals in Malaysian freshwater mussels. Three replicate batches of 5-10 specimens of native *Pilsbryoconcha compressa* and non-native *Sinanodonta woodiana* were collected from one and three habitats, respectively, i.e. a rice paddy channel (both species), a lake and an abandoned mining pool (only *S. woodiana*). Macro- and micronutrient concentrations were determined on freeze-dried, ground mussel meat powder using established methods, including inductively coupled plasma mass spectrometry and direct mercury analyser. Concentrations differed significantly between and within species, but all populations were excellent sources of essential micronutrients. A serving of six mussels on average covered >100% of the recommended daily intake for adults of chromium, iron and manganese, and about 40-60% of calcium, copper, selenium and zinc. However, three of the four populations exceeded permissible levels of some heavy metals, especially arsenic and lead. Protein levels were low with 5-9 g 100 g^{-1} wet weight. Freshwater mussels may therefore represent an important nutrient source for rural, low-income communities, but should not be eaten in large quantities.

Keywords: food analysis; food composition; heavy metals; invertebrates; macronutrients; micronutrients; Mollusca; nutrient deficiency; Unionidae; Unionida

1. Introduction

Southeast Asia has seen a vast improvement in human development over the last few decades, including a steep decrease in poverty and improved access to drinking water (United Nations Development Programme, 2016). However, nutrient deficiency and malnutrition is a persistent problem in the region, which exhibits the world's highest prevalence of inadequate calcium (Ca) and magnesium (Mg) intake (Beal et al., 2017). Even in Malaysia, one of the most developed countries in Southeast Asia, inadequate intake of both macro- and micronutrients is considerable, particularly for Ca (72% prevalence in 2015), but also zinc (Zn) (8%), iron (Fe) (6%), Mg (2%), copper (Cu) (1%) and protein (Khor, 1997; United Nations Development Programme, 2016; Beal et al., 2017). Deficiency in these nutrients can lead to impairment of physical growth and neuropsychological development, as well as a number of diseases, including osteoporosis and anaemia (Fujita, 2000; Hambidge, 2000; Stoltzfus, 2003).

In Malaysia, aboriginal and other rural communities living in the interior of the peninsula and Borneo are particularly vulnerable to nutrient deficiency (Khor, 1997; Sagin et al., 2002; Wong et al., 2014). These communities rely mostly on natural, unfortified foodstuffs collected from the forest, palm plantations, rivers and lakes, including terrestrial mammals, plants and fungi, as well as freshwater fish and shellfish (Dentan, 1991). Scientific knowledge on the nutritional content of these food items is incomplete. Hoe and Siong (1999) showed that most indigenous fruits and vegetables growing in Sarawak's forests are highly nutritious, particularly with respect to energy, protein and potassium (K). Indigenous small freshwater fish species, which are more affordable and accessible to these communities compared to larger fish species, have been shown to be particularly good sources of protein, Fe, Zn and Vitamin A (Kawarazuka and Béné, 2011). However, the nutritional content of other freshwater food sources in this region, such as molluscs and other shellfish, is poorly studied.

Freshwater mussels (order Unionida) are bottom-dwelling, filter-feeding bivalves that are commonly found in lakes and rivers across the globe (Zieritz et al., 2018a and references therein). Malaysia hosts 17 native and one non-native species – the Chinese pond mussel (*Sinanodonta woodiana*), which is now the most widespread and common species in the country (Zieritz et al., 2016, 2018b; Zieritz and Lopes-Lima, 2018). Besides their ecological importance and economic value for ornamental and other purposes (Chowdhury et al., 2016 and references therein), freshwater mussels are regularly collected as a food source by people particularly in Southeast Asia (Hamli et al., 2012; Zieritz et al., 2018a). Due to their filter-feeding life mode, freshwater mussels are also very efficient bioaccumulators and known indicators for metal contamination in the water (Zuykov et al., 2013). In Malaysia, as in many other developing countries, mining of tin, sand, bauxite and other resources has been known to cause particularly high heavy metal concentrations in the water and sediment (Shuhaimi-Othman et al., 2008; Madzin et al., 2015).

A good understanding of nutrient and potentially harmful heavy metal concentrations in freshwater mussels is particularly important for regions where these animals are regularly consumed by people. However, data available in this respect are almost exclusively restricted to Europe, North America and Australia; regions where freshwater mussels are not part of the human diet anymore (Parmalee and Klippel, 1974). Data on nutrient and harmful metal concentrations in natural (sub)tropical freshwater mussel populations are confined to a handful of studies on the macronutrient concentrations of two common genera of Indian freshwater mussels (e.g. Shafakatullah et al., 2013) and heavy metal concentrations in one Vietnamese species (Wagner and Boman, 2004).

This study aims to determine the benefits and risks associated with the consumption of freshwater mussels in Malaysia. This is achieved by determining concentrations of macronutrients, micronutrients and potentially harmful heavy metals across two species and three habitats. The data collected is subsequently used to assess potential dietary intakes, exceedance of legal toxicity thresholds of harmful heavy metals and contributions to estimated daily requirements of macro- and micronutrients.

2. Materials and Methods

2.1. Study sites and sampling of specimens

Freshwater mussels were collected by hand in October/November 2016 from three sites in three different river catchments in Peninsular Malaysia (Fig. 1). Sites were selected to reflect different environmental conditions. All sites are regularly frequented by locals who collect mussels as a source of food.

(1) The rice paddy channel (site "channel"; 4.0844N, 100.9509E) within the River Perak catchment near the village of Kampung Gajah is very narrow (<5 m wide) and shallow (<50 cm depth), with a pH of about 6.3 and specific conductivity of 98 uS/cm (Zieritz et al., 2016). It is inhabited by two freshwater mussel species, i.e. the native *Pilsbryoconcha compressa* (Pc) and the non-native *Sinanodonta woodiana* (Sw) in moderate densities (<50 mussels/m^2).

(2) Semenyih Lake (site "lake"; 2.9475N, 101.8596E) within the Langat River catchment is a eutrophic, man-made lake located in a recreational zone for an adjoining residential area about 3 km from Semenyih, a town of a population of about 90,000. The lake is about 600 m in length, 60,000 m^2 in surface area and reaches a maximum depth of about 2 m. Sw, the only freshwater mussel inhabiting the lake, is present at very high densities of up to 100 individuals/m^2 (Zieritz et al., 2016).

(3) The third site was an abandoned mining pool in the former tin mining area Bestari Jaya (site "pool"; 3.4177N, 101.4441E), which covers an area of 360 ha and is part of the Selangor River catchment (Ashraf et al., 2012). The abandoned mining pool is about 250 m in length, 16,000 m^2 in surface area and 1.5 m in depth, with a pH around 6 and chloride concentrations about 1.5 mg/L (Ashraf et al., 2012). Sw, the only freshwater mussel inhabiting the mining pool, is present at high densities of approximately 50 individuals/m^2 (Zieritz et al., 2016).

At each of the three sites, we randomly selected three spots that were at least 20 m apart from each other. At each of these spots, we collected 5-10 specimens from each mussel species present, resulting in a total of 12 batches of 5-10 specimens each. Mussels were kept alive and held in buckets with water during transportation to the laboratory.

2.2. Preparation of samples in the laboratory

Samples were processed in 12 batches of 5-10 specimens each (see 2.1). On the day of sampling, in the laboratory, mussels were opened by cutting through the adductor muscles. Meat was removed from shells using a scalpel, weighed to ±0.0001 g and freeze-dried (Freeze dryer: Alpha 1-2LDplus, CHRiST, Osterode am Harz, Germany) for 48h to remove water. After freeze-drying, mussel samples were weighed again, ground into powder using a Phillips mixer grinder and sieved through 53 µm mesh size to obtain a homogenous, fine powdered sample. Mussel powders were stored in the 12 batches at -70°C until analysis.

2.3. Macronutrient analyses

Total protein, lipid and ash contents were measured in triplicate for each of the 12 dried samples. Protein content was determined by Kjeldahl method on 0.1 g mussel powder following guidelines by the Association of Official Analytical Chemists (2016) and Sáez-Plaza et al. (2013) using K-415 Scrubber, K-446 KjelDigester, K-360 KJelFlex Distillation unit and Titanium tabs (Buchi, Flawil, Switzerland), H_2SO_4 and NaOH (R&M Chemicals, Semenyih, Malaysia), H_3BO_3 (Merck KGaA, Bandar Sunway, Malaysia) and HCl (Fisher Scientific, Shah Alam, Malaysia). Fat content was determined for 0.05 g samples following Bligh and Dyer (1959) using $CHCl_3$ (Merck KGaA, Bandar Sunway, Malaysia), CH_3OH (RCI Labscan, Cheras, Malaysia), Centrifuge 5430 (Eppendorf, Hamburg, Germany) and Whatman GF-C filter papers. Ash content was determined by incineration of pre-weighed samples (~1 g) at 650°C for 24 h and subsequent weighing of the samples. Carbohydrate content was calculated by difference (total weight – (ash + lipid + protein)).

2.4. Micronutrient and heavy metal analyses

Mussel tissue samples (0.5 g) were digested in HNO_3:10mL/H_2O_2:1mL mixed solution (Romil, Cambridge, UK) in a closed vessel microwave heating system (MARS Xpress, Cambourne, UK) using the protocol of Joy et al. (2015). Subsequent total elemental analysis was carried out by inductively coupled plasma mass spectrometry ((ICP-MS; Agilent 7500cx, Cheadle, UK) using (i) collision cell mode (He-gas) for Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, K, Pb, Sb, Sn, V and Zn; and (ii) H_2-reaction cell mode for selenium (Se) as described by Joy et al. (2015). Total mercury (Hg_T) was measured using a DMA-80 (direct mercury analyser Milestone Inc., Thaxted, UK), using 0.05 g of dried mussel tissue. Samples were weighed into individually pre-cleaned (heated to 550 ^oC for 5 minutes) nickel weighing boats, randomised in batches of 10 and thermally decomposed (using O_2 rich furnace) at 650 °C and quantifiably measured through atomic absorption. Certified reference materials (CRMs) were used for a variety of sample matrices as a check on data quality (Table 1). Limits of detection (LODs) were derived for each element from three blank digests per microwave batch and calculated as 3x standard deviations of these blanks. All samples were handled according to good laboratory practice, for example, avoidance of sample contamination in a clean laboratory environment.

2.5. Statistical analyses and comparison with legal limits and published results

Differences between populations in concentrations of macronutrients, micronutrients and potentially harmful heavy metals were assessed using ANOVA, fitting “population” as a factor with four levels, followed by a Tukey’s posthoc test for pairwise comparisons. Statistical analyses were performed in R version 3.4.1.

Previously published macro- and micronutrient concentrations of other freshwater mussel populations, freshwater (shell)fish and marine bivalves were collected for comparison. We further gathered information on Recommended Daily Intakes (RDIs), Legally Permissible Limits (LPLs) and Provisional maximum tolerable intakes (PMTIs), which were used to calculate the proportion of RDIs covered by a serving of freshwater mussels and identify potential health hazards associated with freshwater mussel consumption. For this purpose, macronutrient and heavy metal content per g mussel tissue was calculated for each of the four mussel populations separately using the average concentration obtained for each population.

3. Results

3.1. Figures of merit of the analysis

All measurements were well above limits of detection (LODs) with the exception of Sb, which was commonly measured as 0.00 µg g^{-1} (Tables 1 and 3). Comparison of measured results from certified reference materials (CRMs) with certified reference values generally indicated good recovery above 90% (Table 1). Mean percent variation of duplicate measurements was relatively high for Al (34 and 94%) and Sn (85 and 102%).

3.2. Description of data, including comparisons between populations and with previously published data

3.2.1. General description of samples

Dry weight per mussel after freeze-drying ranged from 0.07 (Pc) to 5.5 g (Sw), averaging about 2 g dry weight and 10 g wet weight per mussel. Water content ranged from 75 to 85% of wet weight but was visibly highly dependent on the amount of water left inside the valves. In the following, we refer wherever possible to dry weight (DW) data, whilst wet weight (WW) data for all four study populations were calculated assuming an average water content of 80% (i.e. using a conversion factor of 0.2).

Significant differences in concentrations of macro- and micronutrients as well as harmful heavy metals were found both between species at the same location as well as within the same species at different locations, and are presented in detail in the following (Figures 2 and 3, Tables 2 and 3).

3.2.2. Macronutrients (proteins, carbohydrates, lipids)

Protein concentrations of the study populations ranged from about 25-45 g 100 g^{-1} DW or 5-9 g 100 g^{-1} WW (Fig. 2, Table 2). This was within the range of previously reported values from freshwater mussels from India and the USA, which lie between 40 and 51 g 100 g^{-1} DW (Table 2), and was exceeded by freshwater fish from the region by about 15-50 g 100 g^{-1} DW (Puwastien et al., 1999; Bogard et al., 2015) (Table 2). Lipid and ash concentrations, ranging from about 2-10 and 2-25 g 100 g^{-1} DW, respectively, were similar to previously reported values for freshwater mussels and fish (Table 2).

Protein, carbohydrate and ash content differed significantly between the four study populations (ANOVA: protein: $F=22.87$, $df=3$, $P<0.0001$; carbohydrates: $F=9.01$, $df=3$, $P=0.006$; ash: $F=14.4$, $df=3$, $P=0.001$). Protein was significantly higher in populations Pc-channel and Sw-lake compared to Sw-channel and Sw-pool (Fig. 2). Carbohydrate content was highest in the Sw-pool population, and ash content was highest in the two channel populations. No differences were found in the fat contents between mussel populations (ANOVA: $F=0.61$, $df=3$, $P=0.63$).

3.2.3. Micronutrients

Concentrations of seven essential micronutrients with exclusively or predominantly positive effects in humans were in the same range or higher than previously published data on freshwater mussels and other freshwater and marine organisms (Table 3). Ca, Fe and Zn content was particularly high in our study populations. Ca concentrations, ranging from about 16,000-80,000 µg g^{-1} DW, were exceeded only by some *Unio mancus* populations in Lake Maggiore, Italy (Ravera et al., 2003a), and some freshwater fish in Bangladesh (Bogard et al., 2015) (Table 3). Fe concentrations, ranging from 6,000-17,000 µg g^{-1} DW, were exceeded only by some *Hyridella menziesi* populations from the Waikato River, New Zealand (Hickey et al., 1995), but were at least one magnitude lower in freshwater fish (Roos et al., 2007; Bogard et al., 2015). Zn concentrations, ranging from 100-600 µg g^{-1} DW, were exceeded by several other freshwater mussel populations but no other organisms in our dataset (Table 3).

With regard to differences between our study populations, the two rice paddy populations (Pc-channel and Sw-channel) showed significantly higher levels of Ca, Mg and Zn compared to those from the lake and abandoned mining pool (Sw-lake and Sw-pool; Fig. 3). Population Sw-lake was particularly high in K and Mo, whilst Sw-pool was comparatively low in almost all micronutrients (Fig. 3).

3.2.4. Harmful heavy metals

With a maximum observed concentration of 69 µg g^{-1}, lead (Pb) concentrations were exceptionally high in all our study populations when compared to previously published data (Table 3). The same was true for aluminium (Al), though previously published data on freshwater mussels in this respect are restricted to only two studies from Australia and Italy, respectively (Hickey et al., 1995; Ravera et al., 2003a). Arsenic (As) and cadmium (Cd) concentrations were in the same range of those reported in other freshwater mussel populations and exceeded by a few, whilst Hg levels were low (Fig. 3, Table 3). Little to no comparable data were available for antimony (Sb) and tin (Sn) (Table 3).

Sw-pool in general showed the lowest heavy metal concentrations with the exception of Cu, which significantly exceeded levels of Sw-channel and Sw-lake (Fig. 3). Sw-lake showed particularly high levels of Al, As, mercury (Hg) and Sb, whilst Sw-channel was particularly high in Cd and Hg (Fig. 3).

3.3. Estimated dietary intakes and exceedance of legal toxicity thresholds

3.3.1. Macronutrients

Applying an average weight of 2 g DW and 10 g WW per mussel (see section 3.2.1), a serving of six mussels (equalling ~12 g DW and 60 g WW) would on average provide 3.9 g protein and 5.8 g carbohydrates, corresponding to <10% of the recommended daily intake (RDI) for adolescents and adults (Table 4), as well as 0.6 g fat.

3.3.2. Micronutrients

Based on our dataset, a serving of six mussels would on average provide 462 mg Ca, 80 µg chromium (Cr), 390 µg Cu, 119 mg Fe, 29 mg manganese (Mn), 13.5 µg selenium (Se) and 3.1 mg Zn. This corresponds to a contribution of >100% of the RDIs for adolescents and adults of Cr, Fe and Mn, and about 40-60% of Ca, Cu, Se and Zn (Table 4). The contribution for K, Mg and molybdenum (Mo) would be comparatively low with <1-18%.

3.3.3. Harmful heavy metals

Three of the four study populations exceeded the standards set by the Ministry of Health Malaysia (1985) for at least one heavy metal (Fig. 3, Tables 3 and A.1). Specifically, Pc-channel and Sw-lake exceeded levels of As and Pb, and Sw-channel exceeded levels of As and Zn. In addition, standards set in other countries were exceeded by Sw-pool for As, Pb and cobalt (Co), Sw-channel for Pb, and Pc-channel and Sw-lake for Cr (Fig. 3, Tables 3 and A.1). Concentrations of Sb and Sn were far below the maximum permissible level thresholds (Fig. 3, Tables 3 and A.1).

4. Discussion

4.1. Benefits and hazards of consuming freshwater mussels

Ca, Fe and Zn deficiencies are prevalent amongst Malaysians, particularly amongst the lower socio-economic groups (Beal et al., 2017). Ca deficiency is widely known as a cause for osteoporosis, but is also connected with hypertension, diabetes mellitus, neurodegenerative diseases and degenerative joint disease (Fujita, 2000). Fe^{2+} deficiency is the most important contributor of anaemia, causing impaired muscle function and physical performance, as well as being associated with elevated maternal mortality (Stoltzfus, 2003). Due to its importance in subcellular metabolism, inadequate intake of Zn can cause impairment of physical growth and neuropsychological development, and is associated with gastrointestinal and respiratory infections (Hambidge, 2000). Our data indicate that freshwater mussels could be a very important source for these and other micronutrients. On average, a serving of mussels covers >100% of RDIs for adolescents and adults of Fe, 40-60% of Ca and Zn. This is of particular relevance for indigenous and other rural communities, who do not have access to milk products and fortified foods. It should be noted, however, that the proportion of heme-iron in freshwater mussels, which has higher bioavailability than non heme-iron (Taniguchi et al., 2017), has never been assessed. That said, the RDIs by the Ministry of Health Malaysia (2005), which our calculations are based on, presume that 15% of dietary iron is received in heme form. This value is at the lower end of the scale of heme-iron content measured in marine mussels, ranging from about 10 to 60% (Kongkachuichai et al., 2002; Taniguchi et al., 2017), suggesting that freshwater mussels are likely to be an excellent source of heme-iron.

Unfortunately, all our study populations also exhibited very high levels of harmful heavy metals and may therefore present a health hazard. Levels were particularly high for As and Pb. Long-term exposure to As can lead to chronic As poisoning, skin lesion, cancer and a multitude of other dermatological, neurological and gastrointestinal effects (Tchounwou et al., 2004). That said, As is much less toxic in its organic forms, such as arsenosugars, which represent 80-100% of arsenicals in freshwater mussels (Koch et al., 2001; Francesconi and Kuehnelt, 2004; Soeroes et al., 2005; Schaeffer et al., 2006). Chronic exposure to Pb commonly causes anaemia, headache, irritability, lethargy, convulsions, muscle weakness, ataxia, tremors and paralysis (WHO World Health Organization, 2010). In children, even relatively low levels of exposure can cause serious and, in some cases, irreversible neurological damage.

Whilst levels of these and some other heavy metals were high in our study populations and other freshwater mussel populations across the globe (see Table 3), it is unlikely that current levels of consumption are causing health issues. In contrast to rice, fish and vegetables, freshwater mussels are eaten only on an occasional basis rather than being part of the daily diet (Dentan, 1991; A. Zieritz, personal observation). However, provisional maximum tolerable intakes (PMTIs) for adults of 50 kg as set out by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2014) would be exceeded at a regular and/or high intake of freshwater mussels (>14 mussels per week for adults and >7 mussels per week for children; Table 5). This would be expected to cause health problems due to a dietary overload of Fe, and, in some populations, excess Al and/or Pb intake. Especially children should therefore eat freshwater mussels only occasionally and in small portions.

The contribution of freshwater mussels to the human protein diet is probably rather small, as one serving would provide less than 10% of the RDI (Table 4). That said, whilst freshwater mussels may not be a particularly useful source of protein, they are valuable for their lipid and micronutrients they provide.

4.2. Potential causes for variation in nutrient and heavy metal content

Mussels collected from the abandoned mining pool at Bestari Jaya exhibited the lowest levels of all study populations for almost all heavy metals assessed in the present study. It is unlikely that this is due to lower background levels in the soil and water at the site compared to the two other sites, particularly when considering that the site lies within a former tin mining area. We rather suspect that these intraspecific, inter-site differences in heavy metal concentrations might be associated with differences in either feeding and/or age structure of populations. For example, higher availability of suitable food at a site could be expected to lead to lower filtration activity and/or faster growth rate and/or younger maximum age, resulting in a lower concentration of accumulated heavy metals in mussel tissue. Similar mechanisms might be responsible for the significant differences in heavy metal concentrations observed between *P. compressa* vs. *S. woodiana* at the rice paddy channel.

4.3. Importance of freshwater mussel research and conservation

Our study has shown that freshwater mussels may act as an important source of micronutrients, in particular Ca, Fe and Zn, for rural, low-income communities in Southeast Asia. Further studies will be needed to validate our results in other Southeast Asian countries and river basins. In addition, future research on this matter should be concentrated in sub-Saharan Africa, which is the region with the highest prevalence of insufficient nutrition, particularly with regard to Ca, Zn, Se and iodine (I) (Joy et al., 2014, 2015). Freshwater mussels, including the freshwater oyster *Etheria* spp., are commonly consumed in that region (D. Akele, personal communication), but their nutritional content and concentrations of harmful elements is unknown. Data on nutrient and toxin content from various species and habitats should ideally be collected in combination with quantification of intake rates, determination of relative content of particular species of essential and toxic macro- and micronutrients (e.g. Fe2+, inorganic As, Cr(VI)), as well as exposure levels to harmful elements *via* other routes such as drinking water, air or other food sources.

Due to pollution and habitat destruction, many native freshwater mussels are declining and endangered (Zieritz et al., 2016). Of the 17 native Malaysian species, nine are considered threatened by the International Union for Conservation of Nature, four could not be assessed due to a lack of data and only four are considered to persist in healthy population numbers (Zieritz and

Lopes-Lima, 2018). However, in Southeast Asia and many other regions of the world, no legislations to protect endangered freshwater mussel species are in place (Zieritz et al., 2018a). Apart from the importance of mussels to their ecosystems, ecosystem services they provide to people (such as purifying water) and value as pearl producers, the present work has shown that freshwater mussels are valuable as a source of micronutrients for rural populations and safe to be consumed in moderation.

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Conflict of interest

Conflicts of interest: none.

Ethical statement not applicable for this work on invertebrates.

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Figures captions

Figure 1. Location of the three study sites in Peninsular Malaysia. (1) Rice paddy channel in Perak River catchment. (2) Semenyih Lake in Langat River catchment. (3) Abandoned mining pool in Selangor River catchment.

Figure 2. Boxplots of macronutrient concentrations in dry tissues of freshwater mussels sampled in Oct/Nov 2016 from four sites in Peninsular Malaysia, showing medians (bold line), first and third quartiles (box) and 95% confidence intervals of median (whiskers) (n=3, 10-15 mussels per replicate). Different letters above bars indicate statistically significant difference between populations ($p<0.05$). Abbreviations: *Pc*, *Pilsbryoconcha compressa*; *Sw*, *Sinanodonta woodiana*.

Figure 3. Metal concentrations with exclusively/predominantly positive effects (Ca, Fe, K, Mg, Mo, Se, Zn), exclusively negative effects (Al, As, Cd, Hg, Pb, Sb, Sn) and both positive and negative effects (Co, Cr, Cu, Mn, Ni, V) in dry tissues of freshwater mussels sampled in Oct/Nov 2016 from four sites in Peninsular Malaysia, showing medians (bold line), first and third quartiles (box) and 95% confidence intervals of median (whiskers) (n=3, 10-15 mussels per replicate). Different letters above bars indicate statistically significant difference between populations ($p<0.05$). Abbreviations: Pc, *Pilsbryoconcha compressa*; Sw, *Sinanodonta woodiana*.

* exceeding permissible limits set by Malaysian Food Regulation (1985)

[$] exceeding permissible limits set by at least one other national or international standard listed in Table A.4

Table 1. Limit of detection (LOD) and results of duplicate/triplicate measurements of (A) 19 elements obtained by ICP-MS (isotope measured given) and (B) Hg obtained by DMA Hg analyser from Certified Reference Material: BCR-627 (Tuna fish tissue, Merck, Darmstadt, Germany), ERM-BB422 (Fish Muscle, Merck, Darmstadt, Germany), HR1 (River sediment), MESS3 (Marine sediment, Evisa, Canada), TH2 (Lake Sediment). All units in µg g^{-1}. na, data not available.

A	^{27}Al	^{75}As	^{42}Ca	^{111}Cd	^{59}Co	^{52}Cr	^{63}Cu	^{56}Fe	^{24}Mg	^{55}Mn	^{95}Mo	^{60}Ni	^{39}K	^{208}Pb	^{121}Sb	^{78}Se	^{118}Sn	^{51}V	^{66}Zn
LOD	0.4	0.007	15	0.003	0.002	0.02	0.3	2	0.3	2	0.005	0.008	3	0.02	0.01	0.002	0.005	0.02	0.2
BCR-627 x5	14	4.361	414	0.198	0.042	0.276	2.266	70	1034	0.590	0.034	0.442	11450	0.501	0.007	1.778	0.717	0.033	17.480
BCR-627 Dup x5	10	4.497	395	0.215	0.067	0.292	2.310	69	1026	0.722	0.026	1.356	11823	0.491	0.007	1.661	1.781	0.030	17.661
Average	12	4.429	405	0.206	0.054	0.284	2.288	70	1030	0.656	0.030	0.899	11636	0.496	0.007	1.720	1.249	0.031	17.571
mean % variation	34	3	5	8	45	5	2	2	1	20	25	102	3	2	4	7	85	10	1
certified value	na	4.800	na	na	na	na	na	na	na	na	na	na	na	na	na	2.800	na	na	na
%recovery	na	92	na	na	na	na	na	na	na	na	na	na	na	na	na	61	na	na	na
ERM-BB422 x5	1	11.518	316	0.008	0.015	0.175	1.615	10	1423	-0.006	0.015	0.183	17985	0.004	0.007	1.138	0.010	0.009	13.841
ERM-BB422 Dup x5	0	11.932	332	0.011	0.017	0.098	1.635	9	1525	0.176	0.013	0.184	19140	0.001	0.006	1.167	0.003	0.012	14.910
Average	1	11.725	324	0.009	0.016	0.137	1.625	10	1474	0.085	0.014	0.183	18563	0.002	0.006	1.152	0.007	0.010	14.376
mean % variation	94	4	5	34	13	56	1	7	7	214	16	1	6	118	18	3	102	32	7
certified value	na	12.700	330	na	0.015	na	1.670	9.4	1400	0.368	na	na	21700	na	na	1.330	na	na	16.000
%recovery	na	92	98	na	109	na	97	104	105	23	na	na	86	na	na	87	na	na	90

B	Hg
LOD	0.01
MESS3	0.08
MESS3 Dup	0.10
Average	0.09
%difference	21
Expected value	0.091
%recovery	97

HR1	0.33
HR1 Dup1	0.33
HR1 Dup2	0.34
Average	0.34
%difference	4
Expected value	0.342
%recovery	98
TH2	0.63
TH2 Dup1	0.54
TH2 Dup2	0.68
Average	0.61
%difference	22
Expected value	0.620
%recovery	98
BB442	0.59
BB422 Dup1	0.61
BB422 Dup2	0.59
Average	0.60
%difference	3
Expected value	0.601
%recovery	100

Table 2. Macronutrient concentrations (averages or ranges, respectively, in g 100 g^{-1} Dry Weight) of freshwater mussels (Unionida) and fish populations. nd, no data

Species	Protein	Carbohydrates	Lipids	Ash	n	Samples size per replicate	Reference
Pilsbryoconcha compressa (Channel, Malaysia)	36.2	43.3	3.8	16.6	3	5-10 individuals	this study
Sinanodonta woodiana (Channel, Malaysia)	28.2	41.2	6.8	23.8	3	5-10 individuals	this study
Sinanodonta woodiana (Lake, Malaysia)	37.5	51.6	4.8	6.1	3	5-10 individuals	this study
Sinanodonta woodiana (Pool, Malaysia)	27.4	57.7	4.6	10.3	3	5-10 individuals	this study
Actinonaias carinata (USA)	43.3	25.0	3.9	16.7	1	nd	Parmalee & Klippel (1974)
Elliptio dilatata (USA)	28.9	44.7	3.1	23.3	30	5 individuals	Greseth et al. (2003)
Lamellidens marginalis (India)	41.5	40.1	5.1	13.2	nd	nd	Haldar *et al.* (2014)
Lamellidens jenkinsianus (India)	48.8	26.0	6.2	19.0	24	nd	Shafakatullah *et al.* (2013)
Lamellidens generosus (India)	49.4	28.0	6.3	16.5	24	nd	Shafakatullah *et al.* (2013)
Lamellidens marginalis (India)	48.5	28.5	5.4	17.7	24	nd	Shafakatullah *et al.* (2013)
Lampsilis cardium (USA)	24.2	45.2	3.8	26.8	32	5 individuals	Greseth et al. (2003)
Parreysia corrugata (India)	44.4	30.7	5.5	19.5	12	nd	Shafakatullah & Krishnamoorthy (2014)
Parreysia favidens (India)	41.2-60.8	14.8-42.3	3.8-8.2	na	nd	nd	Shetty *et al.* (2013)
Proptera alata (USA)	41.3	33.9	3.5	18.7	1	nd	Parmalee & Klippel (1974)
Quadrula pustulosa (USA)	17.9	61.5	2.7	17.9	32	5 individuals	Greseth et al. (2003)
Radiatula khadakvaslaensis (India)	40.6-57.2	18.3-40.2	3.2-7.6	na	nd	nd	Shetty *et al.* (2013)

Several freshwater fish (Thailand)	51.0-94.9	0	2.8-42.1	3.2-8.1	1 per species	1-2 kg	Puwastien *et al.* (1999)
Several freshwater fish (Bangladesh)	51.9-91.1	0	2.0-43.7	2.3-22.7	1 per species	~2 kg	Bogard *et al.* (2015)
Several freshwater fish (Canada)	80.6-89.4	0	4.9-14.6	4.8-6.8	2-30 per species	10 individuals	Chan *et al.* (1999)

Table 3. Ranges of (heavy) metal concentrations measured in (A) dried and (B) wet tissue [$\mu g\ g^{-1}$] of freshwater and marine organisms.

	Exclusively/predominantly positive effects							Exclusively negative effects						
Population	Ca	Fe	K	Mg	Mo	Se	Zn	Al	As	Cd	Hg	Pb	Sb	Sn
(A) DW														
Freshwater mussels (Unionida)														
Pilsbryoconcha compressa (Channel, Malaysia)	38,900-45,600	6,730-7,060	1,860-2,030	1,500-1,520	0.28-0.38	1.19-1.45	302-313	1,050-1,310	6.8-7.6*	0.69-0.94	0.06-0.08	10.3-69.4*	0.00-0.01	4.57-8.11
Sinanodonta woodiana (Channel, Malaysia)	50,800-79,200	11,400-16,400	1,930-2,030	1,270-1,580	0.33-0.43	0.99-1.25	348-628*	1,040-1,480	9.6-13.0*	1.78-3.35	0.11-0.13	6. 9-9.3[$]	0.00-0.01	4.54-9.18
Sinanodonta woodiana (Lake, Malaysia)	16,600-25,500	8,410-17,200	2,200-2,510	710-766	0.54-0.91	1.11-1.77	107-125	1,860-2,240	22.7-32.0*	0.28-0.47	0.09-0.13	12.1-33.0*	0.01-0.03	0.80-9.87
Sinanodonta woodiana (Pool, Malaysia)	17,400-28,400	6,250-7,680	1,980-2,240	598-688	0.49-0.55	0.40-0.41	97-156	91-160	3.6-4.6[$]	0.09-0.15	0.04-0.06	2. 9-6.9[$]	0.01-0.01	0.90-7.15
Unionidae (Italy)	39,100-74,100				0.5-1.0		361-642*		12-13*	5-10*		11-23*	0.1-0.2	
Unionidae (Poland)		67-78					31-51			0.04-0.08		0.15-0.21		
Anodonta anatina (UK)							152-3500*			0.1-21.1	0.1-11.8*	0.1-45.9*		
Actinonaias carinata (USA)		122	260											
Anodonta cygnea (Iran)										0.01-0.12		0.03-0.26		
Elliptio complanata (USA)											0.64			
Hyridella menziesi (New Zealand)	11,600-26,900	6,620-27,800	1,210-2,140	592-1010		1.9-9.6	164-313	49-363	15-153*	0.21-0.74	0.09-13.1*	0.51-14*		
Pletholophus swinhoei (Vietnam)	5,200-7,600	390-1,900	3,100-3,200			0.5-0.6	120-150		3.4-4.9[$]	0.05-0.08		0.49-0.53		

Proptera alata (USA)		125	410											
Unio mancus (Italy)	989-265,000	166-16,700					136-1,120*	1-708						
Other organisms														
Corbicula javonica (freshwater bivalves; Malaysia)							155-205			0.08-0.62		5.5-11.5*		
Several freshwater gastropods (Malaysia)							0-185		0-225*		0-0.13	0-2.16		
Several marine bivalves (Malaysia)							24-368			0.18-8.51		0.1-19.1*		
Perna viridis (marine bivalve, Malaysia)							75-129			0.68-1.25		2.5-8.8$		
Acetes sp. (marine shrimp, Malaysia)							29-46			0.04-0.55		0.07-0.59		
Several freshwater fish (Cambodia)	20,400-60,400	27-451					40-203							
Macrobrachium vollenhovenii (freshwater prawn, Nigeria)		1.67-3.88					9-15					0.45-1.56		
(B) WW														
Freshwater mussels (Unionida)														
Pilsbryoconcha compressa (Channel, Malaysia)	7,780-9,110	1,350-1,410	372-406	299-304	0.06-0.08	0.24-0.29	61-63	210-262	1.36-1.52*	0.14-0.19	0.01-0.02	2.0-13.9*	0.00-0.00	0.91-1.62
Sinanodonta woodiana (Channel, Malaysia)	10,170-15,900	2,270-3,290	387-407	255-316	0.07-0.09	0.20-0.25	70-126*	208-296	1.91-2.61*	0.36-0.67	0.02-0.03	1.4-1.9$	0.00-0.00	0.91-1.84
Sinanodonta woodiana (Lake, Malaysia)	3,330-5,100	1,680-3,450	440-503	142-153	0.11-0.18	0.22-0.35	21-25	372-448	4.53-6.39*	0.06-0.09	0.02-0.03	2.4-6.6*	0.00-0.01	0.16-1.97

Sinanodonta woodiana (Pool, Malaysia)	3,480-5,690	1,250-1,540	396-449	120-138	0.10-0.11	0.08-0.08	19-31	18-32	0.72-0.93[$]	0.02-0.03	0.01-0.01	0.6-1.4[$]	0.00-0.00	0.18-1.43
Other organisms														
Several freshwater fish (Bangladesh)	93-17,000	4.1-190	580-3,500	210-570		0.05-0.49	6-47							
Several freshwater fish (Canada)	66-598	1.4-6.5				0.26-0.60	4.03-5.81		0.01-0.37	0.00-0.01	0.14-0.53*	0.01-0.03		

*exceeding permissible limits set by Malaysian Food Regulation (1985)

[$] exceeding permissible limits set by at least one other national standards listed in Suppl. Table 3

Table 3 continued

Population	Both positive and negative effects Co	Cr	Cu	Mn	Ni	V	n	Sample size per replicate	Source
DW									
Freshwater mussels (Unionida)									
Pilsbryoconcha compressa (Channel, Malaysia)	0.70-0.75	3.0-12.0$	32.9-70.2	1,530-2,210	1.45-6.92	0.8-1.05	3	5-10 individuals	this study
Sinanodonta woodiana (Channel, Malaysia)	1.09-2.24	2.1-2.3	9.2-14.9	2,200-2,840	1.71-2.36	0.31-0.59	3	5-10 individuals	this study
Sinanodonta woodiana (Lake, Malaysia)	0.46-0.62	7.0-17.4$	7.1-8.5	3,240-3,600	3.26-3.76	0.81-0.9	3	5-10 individuals	this study
Sinanodonta woodiana (Pool, Malaysia)	0.38-0.52	1.9-4.6	12.4-110$	1,070-1,850	0.98-1.83	0.18-0.35	3	5-10 individuals	this study
Unionidae (Italy)	0.7-1	0.4-2	13-34	5,090-11,300	5	0.6-2	1	10 individuals	Ravera et al. (2003b)
Unionidae (Poland)	0.06-0.21	0.3-1.1	4.6-9.3	10-61	0-1.32		1	5 individuals	Rzymski et al. (2014)
Anodonta anatina (UK)			8.2-250$		0.1-45.9		14-16 per population	1 individual	Manly and George (1977)
Actinonaias carinata (USA)							1	na	Parmalee and Klippel (1974)
Anodonta cygnea (Iran)			0.21				3	0.5g DW	Pourang et al. (2010)
Elliptio complanata (USA)							3 per time	Several individuals	Beckvar et al. (2000)
Hyridella menziesi (New Zealand)	0.35-0.83	1.5-2.9	5.8-12.7	1,670-5,110	0.61-1.2		1 per site	8 individuals	Hickey et al. (1995)
Pletholophus swinhoei (Vietnam)		0.14-0.36	2.4-3.2	520-1,600	0.42-0.88		10 per site	1 individual	Wagner and Boman (2004)
Proptera alata (USA)							1	na	Parmalee and Klippel (1974)

Unio mancus (Italy)			4-90	111-32,000			na	na	Ravera et al. (2003a)
Other organisms									
Several freshwater gastropods (Malaysia)			0-115$^{\$}$				na	na	Lau et al. (1998)
Corbicula javonica (Bivalvia, Corbiculidae; Malaysia)			9-19				na	na	Yap and Khairul (2010)
Several marine bivalves, Malaysia)			0.84-36		1.25-7.8		na	na	Hossen et al. (2015)
Perna viridis (marine bivalve, Malaysia)			7.8-20.1				na	na	Yap et al. (2004)
Acetes sp. (marine shrimp, Malaysia)		0.91-1.34	39-56	0.89-1.77			na	8 individuals	Rahouma et al. (2013)
Several freshwater fish (Cambodia)							3	variable	Roos et al. (2007)
Macrobrachium vollenhovenii (freshwater prawn, Nigeria)		0.56-1.33	9.63-13.6				na	na	Jimoh et al. (2011)
WW									
Freshwater mussels (Unionida)									
Pilsbryoconcha compressa (Channel, Malaysia)	0.14-0.15	0.59-2.39$^{\$}$	6.57-14.05	306-443	0.29-1.38	0.16-0.21	3	5-10 individuals	this study
Sinanodonta woodiana (Channel, Malaysia)	0.22-0.45	0.42-0.46	1.84-2.98	439-568	0.34-0.47	0.06-0.12	3	5-10 individuals	this study
Sinanodonta woodiana (Lake, Malaysia)	0.09-0.12	1.39-3.47$^{\$}$	1.42-1.69	648-719	0.65-0.75	0.16-0.18	3	5-10 individuals	this study
Sinanodonta woodiana (Pool, Malaysia)	0.08-0.10	0.37-0.93	2.47-21.90$^{\$}$	214-370	0.20-0.37	0.04-0.07	3	5-10 individuals	this study
Other organisms									

Several freshwater fish (Bangladesh)	0.15-1.2	0.18-23		1 per species	~2 kg	Bogard et al. (2015)
Several freshwater fish (Canada)	0.22-0.37			2-30 per species	10 individuals	Chan et al. (1999)

Table 4. (A) Recommended daily intake and (B) average proportion of RDIs covered by a serving of ~6 freshwater mussels (12g DW or 60g WW) (based on values obtained by the present study)

	Protein	Carbo-hydrates	Ca	Cr	Cu	Fe*	K	Mg	Mn	Mo	Se	Zn
(A) RDIs	[g d^{-1}]	[g d^{-1}]	[mg d^{-1}]	[μg d^{-1}]	[μg d^{-1}]	[mg d^{-1}]	[g d^{-1}]	[mg d^{-1}]	[mg d^{-1}]	[μg d^{-1}]	[μg d^{-1}]	[mg d^{-1}]
Adolescents (10-18 yrs)	45-65[1]	130[2]	1000[1]	21-35[2]	700-890[2]	9-22[1]	4.5-4.7[2]	240-410[2]	1.6-2.2[2]	34-43[2]	23-28[1]	7.5-9[1]
Adults (>19 yrs)	51-65[1]	130[2]	800-1000[1]	20-35[2]	900[2]	9-20[1]	4.7[2]	310-420[2]	1.8-2.3[2]	45[2]	23-33[1]	4.3-6.7[1]
(B) % of RDIs covered by a serving of ~6 mussels based on average concentrations of our dataset												
Adolescents (10-18 yrs)	6-9	4	46	229-382	44-56	542-1330	1	3-6	1310-1790	14-18	48-59	35-41
Adults (>19 yrs)	6-8	4	46-58	229-401	43	596-1330	1	3-4	1250-1600	13	41-59	46-72

[1] Ministry of Health Malaysia (2005)

[2] National Academy of Sciences (USA) (2011)

*assuming 15% bioavailability

Table 5. Provisional maximum tolerable intakes (PMTIs) Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2014) for nine heavy metals and corresponding average intake values for low intake and high intake of freshwater mussels from four populations in Peninsular Malaysia at sites 1-3 (see Fig. 1 for location of sites). Abbreviations: Pc, *Pilsbryoconcha compressa*; Sw, *Sinanodonta woodiana*.

	Al [mg/kg bw/w]	As* [mg/kg bw/w]	Cd [µg/kg bw/m]	Cu [mg/kg bw/d	Fe [mg/kg bw/d]	Hg [µg/kg bw/w]	Pb [mg/kg bw/w]	Sn [mg/kg bw/w]	Zn [mg/kg bw/d]
PMTI	2	0.015	25	0.5	0.8	4	0.025	14	0.3-1
Low intake (6 mussels per week per 50 kg adult)									
Pc1	0.273	0.000	0.813	0.002	0.237	0.016	0.007	0.002	0.011
Sw1	0.305	0.000	2.670	0.000	0.465	0.030	0.002	0.002	0.016
Sw2	0.498	0.001	0.391	0.000	0.403	0.027	0.005	0.001	0.004
Sw3	0.029	0.000	0.118	0.002	0.248	0.012	0.001	0.001	0.005
High intake (30 mussels per week per 50 kg adult)									
Pc1	1.366	0.001	4.065	0.009	**1.184**	0.081	**0.037**	0.008	0.053
Sw1	1.527	0.001	13.350	0.002	**2.326**	0.151	0.010	0.009	0.080
Sw2	**2.490**	0.003	1.955	0.001	**2.015**	0.133	**0.027**	0.004	0.020
Sw3	0.146	0.001	0.590	0.011	**1.242**	0.058	0.007	0.006	0.023
Mussels per day to reach PMTI for 50 kg adult/20 kg child									
Pc1	6/3	76/30	26/11	239/96	3/1.2	213/85	3/1	7640/3050	24/10
Sw1	6/2	47/19	8/3	1090/436	2/1	114/46	11/5	7010/2800	16/6
Sw2	3/1	20/8	55/22	1620/647	2/1	129/51	4/2	14900/5600	64/26
Sw3	59/23	133/53	182/73	188/75	3/1	295/118	15/6	10800/4300	56/22

* assuming a proportion of 10% inorganic (toxic) As in freshwater mussels, which exceeds most of the values obtained to date and is therefore a conservative estimate (Koch et al., 2001; Soeroes et al., 2005; Schaeffer et al., 2006)